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	<i>DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=OR</i>		
<input type="checkbox"/>	L26	L24 and VEGF-C	13
<input type="checkbox"/>	L25	L24 and VEGFR-3	4
<input type="checkbox"/>	L24	530/300.ccls.	3925
<input type="checkbox"/>	L23	L21 and VEGF-C	0
<input type="checkbox"/>	L22	L20 and VEGFR-3	1
<input type="checkbox"/>	L21	L20 and GYWLTIWGY	0
<input type="checkbox"/>	L20	514/9, 11.ccls.	1286
<input type="checkbox"/>	L19	L16 and VEGF-C	6
<input type="checkbox"/>	L18	L16 and VEGFR-3	1
<input type="checkbox"/>	L17	L16 and GYWLTIWGY	0
<input type="checkbox"/>	L16	424/185.1, 192.1.ccls.	2623
<input type="checkbox"/>	L15	L14 and VEGFR-3	14
<input type="checkbox"/>	L14	L12 and dimer	1110
<input type="checkbox"/>	L13	L12 and VEGFR-3	19
<input type="checkbox"/>	L12	(cyclic)adj(peptide)	5247
<input type="checkbox"/>	L11	L8 and antagonist	40
<input type="checkbox"/>	L10	L9 and inhibitor	5
<input type="checkbox"/>	L9	L8 and cyclic	5
<input type="checkbox"/>	L8	L7 and VEGF-C	62
<input type="checkbox"/>	L7	(alitalo)adj(kari)	92
<input type="checkbox"/>	L6	(kari)adj(alitalo)	2
<input type="checkbox"/>	L5	(CGYWLTIWGC)	2
<input type="checkbox"/>	L4	(VEGFR-3)adj(inhibitor)	19
	<i>DB=USPT; PLUR=YES; OP=OR</i>		
<input type="checkbox"/>	L3	5434064.pn.	1
<input type="checkbox"/>	L2	5919638.pn.	1
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L2 0 VEGF RECEPTOR 3 AGONIST

=> s VEGF receptor 3 antagonist
L3 0 VEGF RECEPTOR 3 ANTAGONIST

=> s VEGFR3
L4 341 VEGFR3

=> s L4 and ligand
L5 55 L4 AND LIGAND

=> dup remove 15
PROCESSING COMPLETED FOR L5
L6 28 DUP REMOVE L5 (27 DUPLICATES REMOVED)

=> d 16 1-28 cbib abs

L6 ANSWER 1 OF 28 MEDLINE on STN DUPLICATE 1
2006117086. PubMed ID: 16336962. Signal transduction by VEGF receptors in regulation of angiogenesis and lymphangiogenesis. Shibuya Masabumi; Claesson-Welsh Lena. (University of Tokyo, Institute of Medical Science, 4-6-1 Shirokane-dai, Tokyo 108-8639, Japan.. shibuya@ims.u-tokyo.ac.jp) . Experimental cell research, (2006 Mar 10) Vol. 312, No. 5, pp. 549-60. Electronic Publication: 2005-12-05. Journal code: 0373226. ISSN: 0014-4827. Pub. country: United States. Language: English.

AB The VEGF/VPF (vascular endothelial growth factor/vascular permeability factor) **ligands** and receptors are crucial regulators of vasculogenesis, angiogenesis, lymphangiogenesis and vascular permeability in vertebrates. VEGF-A, the prototype VEGF **ligand**, binds and activates two tyrosine kinase receptors: VEGFR1 (Flt-1) and VEGFR2 (KDR/Flk-1). VEGFR1, which occurs in transmembrane and soluble forms, negatively regulates vasculogenesis and angiogenesis during early embryogenesis, but it also acts as a positive regulator of angiogenesis and inflammatory responses, playing a role in several human diseases such as rheumatoid arthritis and cancer. The soluble VEGFR1 is overexpressed in placenta in preeclampsia patients. VEGFR2 has critical functions in physiological and pathological angiogenesis through distinct signal transduction pathways regulating proliferation and migration of endothelial cells. **VEGFR3**, a receptor for the lymphatic growth factors VEGF-C and VEGF-D, but not for VEGF-A, regulates vascular and lymphatic endothelial cell function during embryogenesis. Loss-of-function variants of **VEGFR3** have been identified in lymphedema. Formation of tumor lymphatics may be stimulated by tumor-produced VEGF-C, allowing increased spread of tumor metastases through the lymphatics. Mapping the signaling system of these important receptors may provide the knowledge necessary to suppress specific signaling pathways in major human diseases.

L6 ANSWER 2 OF 28 CAPLUS COPYRIGHT 2006 ACS on STN
2005:122784 Document No. 142:212747 Fusion proteins of vascular endothelial growth factor homology domains and heparin-binding domains for use as **ligands** of vascular endothelial growth factor receptor 3. Alitalo, Kari; He, Yulong; Tammela, Toumas (Finland). U.S. Pat. Appl. Publ. US 2005032697 A1 20050210, 119 pp., Cont.-in-part of U.S. Ser. No. 669,176. (English). CODEN: USXXCO. APPLICATION: US 2004-868577 20040614. PRIORITY: US 2003-2003/PV47811U 20030612; US 2003-2003/PV47839U 20030612; US 2003-2003/669176 20030923.

AB Synthetic **ligands** for vascular endothelial growth factor receptor 3 that are fusion proteins of a heparin-binding domain and a vascular endothelial growth factor homol. domain and that show greater heparin-binding are described for use in therapeutic control of angiogenesis. These synthetic **ligands** show greater binding to heparin and the **VEGFR3** receptor than its native **ligands**, VEGF-C and VEGF-D. A fusion protein of VEGF-C and the heparin-binding domain of vascular endothelial growth factor was manufactured by expression of the corresponding gene in 293T or 293EBNA cells. The proteins had greater lymphangiogenic activity than VEGF-C in guinea pigs, although the induced blood vessels were leaky.

L6 ANSWER 3 OF 28 MEDLINE on STN DUPLICATE 2
2005115291. PubMed ID: 15561887. Roles of vascular endothelial growth factor receptor 3 signaling in differentiation of mouse embryonic stem cell-derived vascular progenitor cells into endothelial cells. Suzuki

Hiroyuki; Watabe Tetsuro; Kato Mitsuyasu; Miyazawa Keiji; Miyazono Kohei. (Department of Molecular Pathology, Graduate School of Medicine, University of Tokyo, Tokyo 113-0033, Japan.. miyazono-ind@umin.ac.jp) . Blood, (2005 Mar 15) Vol. 105, No. 6, pp. 2372-9. Electronic Publication: 2004-11-23. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

- AB Vascular endothelial growth factor receptor 2 (VEGFR2/Flk-1)-positive cells derived from embryonic stem (ES) cells serve as vascular progenitors, which differentiate into endothelial cells (ECs) in the presence of VEGF-A. **VEGFR3**/Flt-4 (fms-like tyrosine kinase 4) signaling is known to be important for the development of lymphatic endothelial cells (LECs). To elucidate the roles of **VEGFR3** signaling in the differentiation of vascular progenitor cells into ECs, we introduced various types of **VEGFR3** cDNAs into mouse ES cells. VEGF-C, a **ligand** for VEGFR2 and **VEGFR3**, stimulated the endothelial differentiation of the VEGFR2(+) cells transfected with the **VEGFR3** cDNA but not those transfected with kinaseneutral mutants of **VEGFR3**. The **VEGFR3**-transfected ECs exhibited high expression levels of lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1), one of the markers of LECs, and showed efficient binding of hyaluronan. VEGF-C(C152S), which is able to activate **VEGFR3** but not VEGFR2, failed to induce the endothelial differentiation of mock- and **VEGFR3**-transfected VEGFR2(+) cells, suggesting the essential role of VEGFR2 signaling for endothelial differentiation. Furthermore, kinase-negative mutants of **VEGFR3** prevented the VEGF-C-mediated endothelial differentiation of the vascular progenitor cells. Thus, VEGFR2 signaling is required for the endothelial differentiation of mouse ES cells induced by VEGF-C, and **VEGFR3** signaling may confer lymphatic endothelial-like phenotypes to ECs.

L6 ANSWER 4 OF 28 MEDLINE on STN DUPLICATE 3
2005515970. PubMed ID: 16189151. Augmented expression of secondary lymphoid tissue chemokine and EBI1 **ligand** chemokine in Crohn's disease. Kawashima D; Oshitani N; Jinno Y; Watanabe K; Nakamura S; Higuchi K; Arakawa T. (Department of Gastroenterology, Osaka City University Graduate School of Medicine, 1-4-3, Asahi-machi, Abeno-ku, Osaka 545-8585, Japan.) Journal of clinical pathology, (2005 Oct) Vol. 58, No. 10, pp. 1057-63. Journal code: 0376601. ISSN: 0021-9746. Pub. country: England: United Kingdom. Language: English.

- AB BACKGROUND: A dominant T helper type 1 (Th1) immune response is thought to be involved in Crohn's disease (CD). SLC/CCL21 and ELC/CCL19, chemokines that regulate T cell homing and promote recirculating T and dendritic cell (DC) interactions, help control antigen specific T cell responses. AIMS: To investigate the Th1 response and SLC and ELC in CD pathogenesis. METHODS: Surgically resected intestine and mesenteric lymph nodes (MLNs) from controls and patients with CD and ulcerative colitis (UC) were investigated. CD3, CD83, HECA452, **VEGFR3**, SLC, ELC, and CCR7 expression was studied immunohistochemically. CCR7 mRNA was quantified using real time RT-PCR. RESULTS: ELC was almost undetectable in intestinal samples. SLC was found sporadically in lymphoid follicles, lymphoid aggregate venules, and lymphatic vessels. In MLNs, SLC was highly expressed in high endothelial venules (HEVs), lymphatic vessels, and stromal DCs, predominantly in T cell areas. ELC was highly expressed in mature DCs. There were significantly more SLC positive HEVs and ELC positive mature DCs, important components of T cell areas, in CD. SLC, ELC, and CCR7 mRNA was significantly higher in CD MLNs compared with UC. CD MLNs had increased expression of SLC and ELC, mainly in HEVs, mature DCs, and lymphatic vessels, inducing T cell hyperplasia. CCR7 mRNA was increased in T cell areas. CONCLUSION: The dominant Th1 immune response is facilitated by interaction of SLC positive HEVs/lymphatic vessels, ELC positive mature DCs, and CCR7 positive T cells in hyperplastic T cell areas. In CD, memory T cells and mature DCs may home to MLN.

L6 ANSWER 5 OF 28 CAPLUS COPYRIGHT 2006 ACS on STN

2005:1160788 Chemokines in inflammatory bowel disease. Danese, S.; Gasbarrini, A. (Dep. Internal Med., Gemelli Hospital, Catholic Univ., Rome, I-00168, Italy). Journal of Clinical Pathology, 58(10), 1025-1027 (English) 2005. CODEN: JCPAAK. ISSN: 0021-9746. Publisher: BMJ Publishing Group.

AB The study of Kawashima et al. (2005) entitled "Augmented expression of secondary lymphoid tissue chemokine (SLC) and EB11 **ligand** chemokine (ELC) in Crohn's disease" is reviewed with commentary and refs. Kawashima et al. found that Crohn's disease mesenteric lymph nodes show increased expression of both SLC and ELC by immunohistochem. Furthermore, the cells displaying intense immunoreactivity were identified as the endothelial venules, dendritic cells, and lymphatic vessels, because of their colocalization with the specific markers HECA452, CD83, and **VEGFR3**, resp.

L6 ANSWER 6 OF 28 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

2006:208237 Document No.: PREV200600209965. Enhanced VEGFR-3 and Lyve-1 expression despite decreased VEGF-C and VEGF-D expression in duodenal mucosa of idiopathic lymphangiectasia with enteric protein-loss. Hokari, Ryota; Kitagawa, Noritake; Tsuzuki, Yoshikazu; Kato, Shingo; Kawaguchi, Atsushi; Nagao, Shigeaki; Kurihara, Chic; Okada, Yoshikiyo; Alitalo, Kari; Miura, Soichiro. Gastroenterology, (APR 2005) Vol. 128, No. 4, Suppl. 2, pp. A188.

Meeting Info.: Annual Meeting of the American-Gastroenterological-Association/Digestive-Disease-Week. Chicago, IL, USA. May 14 -19, 2005. Amer Gastroenterol Assoc.

CODEN: GASTAB. ISSN: 0016-5085. Language: English.

AB Background: Vascular endothelial growth factor receptor-3 (VEGFR-3), a receptor protein tyrosine kinase and LYVE-1, a hyaluronan receptor, are specifically expressed in the adult lymphatic endothelium. On the other hand VEGF-C and D, **ligands** for VEGFR-3, induce selective hyperplasia of the lymphatic vasculature and are known to play central roles in lymphangiogenesis. In this study, we investigated the expression of VEGFR-3, LYVE-I and VEGF-C/D in the duodenal mucosa of idiopathic intestinal lymphangiectasia. Methods: Tissue samples were obtained from duodenal biopsies in patients With idiopathic intestinal lymphangiectasia complicated with protein-losing with informed consent, Biospies were taken from white spot lesions. As controls biopsy specimens were obtained from healthy subjects. For immunohistochemical analysis, antibodies against human **VEGFR3** and LYVF-1 were used as lymphatic markers. Anti-endothelium (PAL-E) and anti-CD34 antibodies were used to identify venular endothelium. Messenger RNA expression of VEGF-C, VEGF-D, and VEGFR-3 in the mucosa was determined by quantitative PCR method. mRNA expressions of other markers for lymphatic vessel development, Prox 1 and FOXC2 were also examined, Results: In the control mucosa **VEGFR3** was only weakly expressed on the central lymphatic vessels in the lamina propria of intestine and LYVE-1 was expressed mainly on the lymphatic vessels in the submucosa. PAL-E and CD34 were expressed within the venules just below the epithelial cell layer of duodenal villi. On the other hand in the mucosa of intestinal lymphangiectasia, **VEGFR3** and LYVE-1 expression was increased and the intense expression site appeared to correspond to the widely dilated central lymphatic vessels. Messenger RNA expression study showed a significant increase in **VEGFR3** in lymphangiectasia. However, the expression of VEGF-C and VEGF-D mRNA were significantly suppressed despite of the presence of lymphangiectasia compared with controls. mRNA expression of other markers for lymph development, Prox 1 and FOXC2 were also decreased. Conclusions: The present results suggest that there is an increased growth of lymphatic endothelial cells through VEGFR-3 and LYVE-1 receptors in dilated central lymphatic vessels of idiopathic lymphangiectasia, although expressions of lymphangiogenic growth factors are decreased. There is a possibility that there is a dysregulation of lymphangiogenesis which may be closely related

to the pathophysiology of this disease.

L6 ANSWER 7 OF 28 CAPLUS COPYRIGHT 2006 ACS on STN

2005:24285 Document No. 142:296527 Complete and Specific Inhibition of Adult Lymphatic Regeneration by a Novel VEGFR-3 Neutralizing Antibody. Pytowski, Bronislaw; Goldman, Jeremy; Persaud, Kris; Wu, Yan; Witte, Larry; Hicklin, Daniel J.; Skobe, Mihaela; Boardman, Kendrick C.; Swartz, Melody A. (Molecular and Cellular Biology, ImClone Systems, New York, NY, USA). Journal of the National Cancer Institute, 97(1), 14-21 (English) 2005. CODEN: JNCIEQ. ISSN: 0027-8874. Publisher: Oxford University Press.

AB Background: New lymphatic growth may contribute to tumor metastasis. Activation of vascular endothelial growth factor receptor 3 (VEGFR-3) by its **ligands** VEGF-C and -D is necessary for embryonic and tumor lymphangiogenesis. However, the exact role of VEGFR-3 signaling in adult lymphangiogenesis and in lymphatic vessel survival and regeneration is unclear. Methods: A novel rat monoclonal antibody to murine VEGFR-3, mF4-31C1, which potently antagonizes the binding of VEGF-C to VEGFR-3, was developed. We tested the effects of systemic mF4-31C1 administration in a mouse tail skin model of lymphatic regeneration, either with or without local overexpression of VEGF-C, and we observed lymphatic and blood vessel regeneration over time using microlymphangiog. and immunostaining. Results: Normal mice regenerated complete and functional lymphatic vessels within 60 days of surgery. In athymic mice implanted with VEGF-C-overexpressing human breast carcinoma cells, lymphatic regeneration took place over 25 days and resulted in hyperplastic vessels. Under either condition, no lymphatic regeneration occurred in mice receiving mF4-31C1 during the regeneration period. Blood angiogenesis and preexisting lymphatic vessels were unaffected, both in morphol. and in function. Conclusions: Blocking VEGFR-3 completely and specifically prevented both physiol. normal and tumor VEGF-C-enhanced lymphangiogenesis in the adult mouse but had no effect on either blood angiogenesis or the survival or function of existing lymphatic vessels. Thus, targeting VEGFR-3 with specific inhibitors may block new lymphatic growth exclusively.

L6 ANSWER 8 OF 28 CAPLUS COPYRIGHT 2006 ACS on STN

2004:905883 Document No. 141:361107 Methods for the detection of cell surface receptor complexes as cancer biomarkers and therapeutic effectiveness of cleavage thereof. Chan-Hui, Po-Ying; Salimi-Moosavi, Hossein; Shi, Yining; Singh, Sharat; Dua, Rajiv; Mukherjee, Ali; Pidaparathi, Sailaja (Aclara Biosciences, Inc., USA). PCT Int. Appl. WO 2004092353 A2 20041028, 106 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2004-US9717 20040330. PRIORITY: US 2003-2003/PV45988U 20030401; US 2003-2003/623057 20030717; US 2003-2003/PV49448U 20030811; US 2003-2003/PV50803U 20031001; US 2003-2003/PV51294U 20031020; US 2003-2003/PV523258 20031118.

AB The invention is directed to a new class of biomarker in patient samples comprising dimers of cell surface membrane receptors. In one aspect, the invention includes a method of determining the status of a disease or healthful condition by correlating such condition to amts. of one or more dimers of cell surface membrane receptors measured directly in a patient sample, in particular a fixed tissue sample. In another aspect, the invention includes a method of determining a status of a cancer in a specimen from an individual by correlating measurements of amts. of one or more dimers of cell surface membrane receptors in cells of the specimen to such status,

including presence or absence of a pre-cancerous state, presence or absence of a cancerous state, prognosis of a cancer, or responsiveness to treatment. Preferably, methods of the invention are implemented by using sets of binding compds. having releasable mol. tags that are specific for multiple components of one or more types of receptor dimers. After binding, mol. tags are release and separated from the assay mixture for anal.

L6 ANSWER 9 OF 28 CAPLUS COPYRIGHT 2006 ACS on STN

2004:249186 Document No. 140:264484 Screening for inhibitors of vascular endothelial growth factor receptor 3 for use as inhibitors of metastasis or tumor-induced lymphangiogenesis. Krishnan, Jaya; Sleemann, Jonathan (Forschungszentrum Karlsruhe GmbH, Germany). Ger. Offen. DE 10242663 A1 20040325, 11 pp. (German). CODEN: GWXXBX. APPLICATION: DE 2002-10242663 20020913.

AB A method of identifying inhibitors of tumor metastasis formation or tumor-induced lymphangiogenesis that specifically inhibit activation in vivo of the lymphangiogenesis regulating cell receptor vascular endothelial growth factor receptor VEGFR-3 is described. The method involves using cells expressing a gene for the vascular endothelial growth factor C derivative Δ NACVEGF-C/Cys152Ser. This variant can induced angiogenesis and metastasis in weakly metastasizing cell lines and is specific for VEGFR-3. Testing can be carried out in immune-competent animal hosts.

L6 ANSWER 10 OF 28 MEDLINE on STN DUPLICATE 4

2004406503. PubMed ID: 15215251. Vascular endothelial growth factor (VEGF)-D and VEGF-A differentially regulate KDR-mediated signaling and biological function in vascular endothelial cells. Jia Haiyan; Bagherzadeh Azadeh; Bicknell Roy; Duchon Michael R; Liu Dan; Zachary Ian. (Department of Medicine, The Rayne Institute, University College London, 5 University Street, London WC1E 6JJ, UK.) The Journal of biological chemistry, (2004 Aug 20) Vol. 279, No. 34, pp. 36148-57. Electronic Publication: 2004-06-23. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Vascular endothelial growth factor (VEGF)-D binds to VEGF receptors (VEGFR) VEGFR2/KDR and **VEGFR3**/Flt4, but the signaling mechanisms mediating its biological activities in endothelial cells are poorly understood. Here we investigated the mechanism of action of VEGF-D, and we compared the signaling pathways and biological responses induced by VEGF-D and VEGF-A in endothelial cells. VEGF-D induced KDR and phospholipase C-gamma tyrosine phosphorylation more slowly and less effectively than VEGF-A at early times but had a more sustained effect and was as effective as VEGF-A after 60 min. VEGF-D activated extracellular signal-regulated protein kinases 1 and 2 with similar efficacy but slower kinetics compared with VEGF-A, and this effect was blocked by inhibitors of protein kinase C and mitogen-activated protein kinase kinase. In contrast to VEGF-A, VEGF-D weakly stimulated prostacyclin production and gene expression, had little effect on cell proliferation, and stimulated a smaller and more transient increase in intracellular $[Ca^{2+}]$. VEGF-D induced strong but more transient phosphatidylinositol 3-kinase (PI3K)-mediated Akt activation and increased PI3K-dependent endothelial nitric-oxide synthase phosphorylation and cell survival more weakly. VEGF-D stimulated chemotaxis via a PI3K/Akt- and endothelial nitric-oxide synthase-dependent pathway, enhanced protein kinase C- and PI3K-dependent endothelial tubulogenesis, and stimulated angiogenesis in a mouse sponge implant model less effectively than VEGF-A. VEGF-D-induced signaling and biological effects were blocked by the KDR inhibitor SU5614. The finding that differential KDR activation by VEGF-A and VEGF-D has distinct consequences for endothelial signaling and function has important implications for understanding how multiple **ligands** for the same VEGF receptors can generate **ligand**-specific biological responses.

L6 ANSWER 11 OF 28 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN

2004419833 EMBASE Kinase inhibition with BAY 43-9006 in renal cell carcinoma. Ahmad T.; Eisen T.; Yang J.; Atkins M.; Stadler W.. Dr. T. Eisen, Royal Marsden Hospital, Institute of Cancer Research, Downs Road, Sutton, Surrey SM2 5PT, United Kingdom. tim.eisen@icr.ac.uk. Clinical Cancer Research Vol. 10, No. 18 II, pp. 6388s-6392s 15 Sep 2004.

Refs: 24.

ISSN: 1078-0432. CODEN: CCREF4

Pub. Country: United States. Language: English. Summary Language: English. Entered STN: 20041018. Last Updated on STN: 20041018

AB BAY 43-9006 is an oral inhibitor of CRAF, wild-type BRAF, mutant V599E BRAF, vascular endothelial growth factor receptor (VEGFR) 2, **VEGFR3**, mVEGFR2, FLT-3, platelet-derived growth factor receptor, p38, and c-kit among other kinases. A Phase I study of BAY 43-9006 identified 400 mg orally twice daily as the recommended Phase II dose. The Phase II results of a study of BAY 43-9006 at 400 mg orally twice daily were particularly interesting in patients with renal cell carcinoma. Data from the first 41 patients with renal cell carcinoma showed that 30% of patients had stable disease (defined as between 25% reduction and 25% growth), 40% had responded (defined as >25% reduction), and 30% had progressed. Disease could be stabilized for periods in excess of a year. Some lesions became cystic and could actually enlarge while developing a low attenuation core. This phenomenon is recognized in the treatment of gastrointestinal stromal tumors with imatinib mesylate. The toxic effects of BAY 43-9006 were manageable and included hypertension, edema, diarrhea, hand and foot syndrome, rash, and hair loss where the rash involved the scalp. There was an impression of tachyphylaxis such that patients who required a dose reduction could be restored to full dose after a few months. A Phase III randomized, placebo-controlled trial of BAY 43-9006 has started for patients whose renal cell carcinoma has progressed within 6 months of immunotherapy. Combination studies with interferon, interleukin 2, bevacizumab, and chemotherapy are under consideration. The therapeutic targets of BAY 43-9006 in renal cell carcinoma remain unclear. Unlike melanoma, BRAF mutations have not been found in renal cell carcinoma. Other candidate targets include VEGFR2 and **VEGFR3**.

L6 ANSWER 12 OF 28 CAPLUS COPYRIGHT 2006 ACS on STN

2004:834554 Document No. 141:361077 Heterodimerization with vascular endothelial growth factor receptor-2 (VEGFR-2) is necessary for VEGFR-3 activity. Alam, Antoine; Herault, Jean-Pascal; Barron, Pauline; Favier, Benoit; Fons, Pierre; Delesque-Touchard, Nathalie; Senegas, Isabelle; Laboudie, Patricia; Bonnin, Jacques; Cassan, Cecile; Savi, Pierre; Ruggeri, Bruce; Carmeliet, Peter; Bono, Francoise; Herbert, Jean-Marc (Sanofi-Synthelabo Research, Cardiovascular Department, Toulouse, Fr.). Biochemical and Biophysical Research Communications, 324(2), 909-915 (English) 2004. CODEN: BBRC9. ISSN: 0006-291X. Publisher: Elsevier.

AB VEGFR-3 is essential for vascular development and maintenance of lymphatic vessel's integrity. Little is known about its cooperative effect with other receptors of the same family. Contrary to VEGFR-2, stimulation of VEGFR-3 by VEGF-C and -D failed to enhance its phosphorylation either in HEK293T or in PAE cells. These **ligands** were unable to induce angiogenesis of PAEC expressing VEGFR-3 alone. In the presence of VEGFR-2, VEGF-C and -D induced heterodimerization of VEGFR-3 with VEGFR-2. This heterodimerization was associated with enhanced VEGFR-3 phosphorylation and subsequent cellular responses as evidenced by the formation of capillary-like structures in PAE cells and proliferation of primary human endothelial cells expressing both receptors. Taken together, these results show for the first time that VEGFR-3 needs to be associated to VEGFR-2 to induce **ligand**-dependent cellular responses.

L6 ANSWER 13 OF 28 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

2004:683725 The Genuine Article (R) Number: 840CJ. Expression of a flt-4 (**VEGFR3**) splicing variant in primary human prostate tumors. VEGF D and flt-4t(Delta 773-1081) overexpression is diagnostic for sentinel lymph node metastasis. Stearns M E (Reprint); Wang M; Hu Y J; Kim G; Garcia F U . Drexel Univ, Dept Pathol, 15th & Vine Sts, MS 435, Philadelphia, PA 19085 USA (Reprint); Drexel Univ, Coll Med, Dept Pathol & Lab Med, Philadelphia, PA 19104 USA. mark.stearns@drexel.edu. LABORATORY INVESTIGATION (JUN 2004) Vol. 84, No. 6, pp. 785-795. ISSN: 0023-6837. Publisher: NATURE PUBLISHING GROUP, 345 PARK AVE SOUTH, NEW YORK, NY 10010-1707 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Utilizing a cDNA expression library established from human prostate PC-3ML tumor cells, we have cloned a truncated fit-4 gene, termed fit-4t(Delta773-1081). We have then utilized RNase protection and ELISA to measure the relative levels of VEGF B, C, D and fit-1, KDR, fit-4 and fit-4t(Delta773-1081) expression in freshly isolated benign prostatic hyperplasia or BPH tissue (n=21), primary prostate cancers (n=82) and matching sentinel lymph node metastases from stage T2a-T2b/T3 tumors (n=52). Comparisons of the primary tumors with BPH showed that there was a significant upregulation of VEGF-B (P=0.003), VEGF D (P=0.005), fit-1 (P=0.003), KDR (P=0.002), fit-4 (P=0.007), and flt-4t(Delta773-1081) (P=0.001), but not VEGF-C (P=0.543). There was no correlation between VEGF-B and its receptor fit-1 (P=0.545), or VEGF-C and flt-4 (P=0.16) and KDR (P=0.23) receptor expression in tumor specimens. Conversely, there was no significant relationship between VEGF-D and the flt-4t(Delta773-1081) receptor (P=0.516) expression. Statistical analysis further showed that there was no significant correlation between VEGF-B, VEGF-C, VEGF-D, fit-1, KDR, fit-4 and flt-4t(Delta773-1081), with patient age (P>0.10), stage (P> 0.10), PSA value (P> 0.15) or tumor size (P> 0.15). Likewise, there was no significant correlation between VEGF-B, VEGF-C, fit-1, KDR, and fit-4 with Gleason score (P>0.15). In comparison, flt-4t(Delta773-1081), levels clearly increased significantly in Gleason score 7 and Gleason score 8-10 tumors as well as in stage T2a-T2b/T3 tumors. The studies were extended to compare gene expression profiles in T2a-T2b and T3 tumors with (n=26) and without (n=26) matching sentinel lymph node metastases. The data showed that VEGF D and flt-4t(Delta773-1081) expression levels were significantly elevated in primary tumors with sentinel lymph node involvement compared to those lacking lymph node involvement (P>0.0022 and 0.006, respectively). These data suggest that targeting VEGF D and flt-4t(Delta773-1081), receptors may be particularly effective in the prevention of lymph node metastases.

L6 ANSWER 14 OF 28 CAPLUS COPYRIGHT 2006 ACS on STN

2004:1076320 Document No. 142:20823 Extracellular matrix regulates endothelial functions through interaction of VEGFR-3 and integrin $\alpha 5\beta 1$. Zhang, Xuefeng; Groopman, Jerome E.; Wang, Jian Feng (Division of Experimental Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA). Journal of Cellular Physiology, Volume Date 2005, 202(1), 205-214 (English) 2004. CODEN: JCLLAX. ISSN: 0021-9541. Publisher: Wiley-Liss, Inc..

AB Endothelium extracellular matrix (ECM) interactions can provide distinct spatial and mol. signals which control cellular proliferation, migration, and differentiation. Here, we investigated the role of fibronectin (FN), a major ECM protein, on the functions of lymphatic endothelial cells (LEC). We observed that FN, the **ligand** for integrin $\alpha 5\beta 1$, selectively promoted the growth of LEC as compared with vitronectin (VN) in the presence of the **ligand** for vascular endothelial growth factor receptor 3 [VEGFR-3 (VEGF-C156S)]. Upon investigating the mechanisms whereby ECM components regulate VEGFR-3 signaling, we found that FN transactivated VEGFR-3 and significantly enhanced the phosphorylation of VEGFR-3 induced by VEGF-C156S as compared to VN. An enhanced association of the integrin subunit $\alpha 5$ or $\beta 1$ with VEGFR-3, after stimulation with VEGF-C156S, was observed by

co-immunopptn. While blockade of integrin $\alpha 5\beta 1$ inhibited the VEGF-C156S-induced phosphorylation of VEGFR-3, no similar effect was obtained by blocking integrin $\alpha v\beta 3$. FN also protected the endothelial cells from serum deprivation-induced apoptosis. Moreover, while the specific PI3 kinase inhibitor, LY294002, abolished this FN-mediated cell survival, the MAPK kinase inhibitor, PD98059, had no significant effect. Furthermore, a dominant-neg. mutant of VEGFR-3 (G857R) reduced VEGF-C156S or FN-mediated cell survival, as well as the activities of PI3 kinase/Akt. Our results indicate that integrin $\alpha 5\beta 1$ participates in the activation of both VEGFR-3 and its downstream PI3 kinase/Akt signaling pathway, which is essential for FN-mediated lymphatic endothelial cell survival and proliferation.

L6 ANSWER 15 OF 28 CAPLUS COPYRIGHT 2006 ACS on STN

2004:591422 Document No. 141:204543 Immunodetection and quantification of vascular endothelial growth factor receptor-3 in human malignant tumor tissues. Bando, Hiroko; Brokelmann, Maren; Toi, Masakazu; Alitalo, Kari; Sleeman, Jonathan P.; Sipos, Bence; Groene, Hermann-Josef; Weich, Herbert A. (Department of Gene Regulation and Differentiation, National Research Centre for Biotechnology (GBF), Braunschweig, Germany). International Journal of Cancer, 111(2), 184-191 (English) 2004. CODEN: IJCNAW. ISSN: 0020-7136. Publisher: Wiley-Liss, Inc..

AB Vascular endothelial growth factor receptor-3 (VEGFR-3) and its **ligands**, vascular endothelial growth factor-C (VEGF-C) and -D (VEGF-D), are the major mols. involved in developmental and pathol. lymphangiogenesis. Here the authors describe for the first time the development of a specific indirect ELISA for the quantification of VEGFR-3 in different human cell and tissue lysates. A combination of the goat polyclonal anti-VEGFR-3 antibody and the mouse monoclonal anti-human VEGFR-3 antibody was used. The assay was highly sensitive and reproducible with a detection range of 0.2-25 ng/mL. The assay was specific for VEGFR-3, with no cross-reactivity to VEGFR-1 or VEGFR-2. Complex formation with VEGF-C and VEGF-D had no effect on the sensitivity of the assay. The VEGFR-3 concentration in the lysates of cultured human dermal

microvascular endothelial cells was 14-fold higher than in the lysates from human umbilical vein endothelial cells. In human kidney, breast, colon, gastric and lung cancer tissues the protein levels of VEGFR-3 were in the range of 0.6-16.7 ng/mg protein. Importantly, the level of VEGFR-3 protein detected in the ELISA correlated significantly with the number of VEGFR-3 pos. vessels observed in histochem. sections, suggesting that the ELISA assay may be a reliable surrogate of measuring VEGFR-3-pos. vessel d. The protein levels of VEGFR-3 in 27 renal cell carcinoma samples had a significant correlation with the levels of VEGF-C, or biol. active, free VEGF-A, but not with VEGFR-I or total VEGF-A. This assay provides a useful tool for the investigations of the expression levels of VEGFR-3 in physiol. and pathol. processes, particular in cancer and in lymphangiogenesis-related disease.

L6 ANSWER 16 OF 28 CAPLUS COPYRIGHT 2006 ACS on STN

2003:778056 Document No. 139:303788 Method for identification of kinase inhibitors using covalent tethering of **ligands** to kinase locked in inactive conformation. Prescott, John C.; Braisted, Andrew (Sunesis Pharmaceuticals, Inc., USA). PCT Int. Appl. WO 2003081210 A2 20031002, 260 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US8725 20030320. PRIORITY: US 2002-2002/PV366892 20020321.

AB The invention concerns the identification of protein kinase inhibitors that preferentially bind to the inactive conformation of a target protein kinase. The inhibitors are identified by locking the target protein kinase in an inactive conformation, and using a covalent tethering approach to identify inhibitors preferentially targeting the inactive conformation. This method identifies inhibitors which do not compete directly with ATP for binding to the active conformation of the ATP-binding pocket of the kinase. Thus, using the covalent tethering approach to identify small mol. inhibitors, smaller drug-like fragments (monophores) are first tested for binding activity to kinases which have been modified to contain a tether, or which already contain a tether (a cysteine side-chain SH group, for example). These monophores are then used to synthesize conjugates that bind to non-overlapping sites to generate diaphores that no longer require the tether for binding. Merging of multiple fragments in this way results in a combination of individual binding energies plus a favorable entropic term due to the high local concentration of the second fragment once the first fragment is bound thus yielding **ligands** having dissociation consts. in the μM range. This "screen then link" strategy is much more efficient than the traditional approach, allowing a much large survey of chemical diversity space than is achievable using even the largest compound libraries.

L6 ANSWER 17 OF 28 MEDLINE on STN DUPLICATE 5
2003258561. PubMed ID: 12784238. Vascular endothelial growth factor C and vascular endothelial growth factor receptor 3 expression in squamous cell carcinomas of the head and neck. Neuchrist Csilla; Erovic Bohan M; Handisurya Allesandra; Fischer Michael B; Steiner Georg E; Hollemann David; Gedlicka Claudia; Saaristo A; Burian Martin. (Univ. Klinik fuer Hals-Nasen und Ohrenheilkunde, Allgemeines Krankenhaus der Stadt Wien, Waehringer Guertel 18-20, 1090 Wien, Austria.. csilla.neuchrist@univie.ac.at) . Head & neck, (2003 Jun) Vol. 25, No. 6, pp. 464-74. Journal code: 8902541. ISSN: 1043-3074. Pub. country: United States. Language: English.

AB BACKGROUND AND METHODS: VEGF proteins and their receptors are involved in tumor vessel neoformation. The third VEGF receptor, **VEGFR3** (flt-4) is important during both blood vessel development and lymphatic vessel formation. Because HNSCC preferentially metastasizes to regional lymph nodes, we investigated the expression of **VEGFR3** and its **ligand** VEGF-C in head and neck squamous cell carcinomas by semiquantitative RT-PCR (4 HNSCC cells lines and 6 HNSCC specimens) and by immunohistochemistry (18 HNSCC specimens). **VEGFR3** protein expression was confirmed by Western blotting in four HNSCC cell lines and six HNSCC specimens. RESULTS: Semiquantitative mRNA analysis showed VEGF-C mRNA expression in three (SCC9, SCC25, LFFR) of four HNSCC cell lines and all six HNSCC specimens. **VEGFR3** mRNA was found in two HNSCC cell lines (JPPA and SCC25) and only weakly detected in the other two HNSCC cell lines (SCC9 and LFFR). High amounts of **VEGFR3** mRNA were shown in all six patients' tumor specimens. **VEGFR3** Western blot analysis yielded a distinct band at the predicted size of 210 kD in JPPA and SCC9 and hardly detectable bands in SCC25 and LFFR cell lines. All six HNSCC specimens displayed strong **VEGFR3** protein bands. Immunohistochemistry in 18 HNSCC specimens assigned strong to mediate VEGF-C IR and minor **VEGFR3** IR to tumor cells and strong VEGF-C and **VEGFR3** IR to tumor surrounding vessels. In addition, intense VEGF-C immunostaining was observed on perivascular and mononuclear cells in the tumor surrounding stroma. Subtyping of **VEGFR3+** microvascular tumor vessels revealed partially double immunolabeling with CD34 and flk-1, indicating a common origin of blood and lymphatic vessels. The expression of VEGF-C on tumor cells could be correlated with recurrences, and larger primary tumors had more VEGF-C-positive vessels. CONCLUSIONS: The broad expression of VEGF C and **VEGFR3** in HNSCC suggests involvement in tumor lymph angiogenesis and vascular angiogenesis, promoting tumor growth and propagation of cancer cells.

This implies that inhibitors of lymph angiogenesis could become effective therapeutic options similar to classical angiogenesis inhibitors.
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L6 ANSWER 18 OF 28 CAPLUS COPYRIGHT 2006 ACS on STN

2003:601858 Document No. 139:228632 Novel expression of vascular endothelial growth factor receptor (VEGFR)-3 and VEGF-C on corneal dendritic cells. Hamrah, Pedram; Chen, Lu; Zhang, Qiang; Dana, M. Reza (Laboratory of Immunology, Schepens Eye Research Institute and the Massachusetts Eye and Ear Infirmary and Department of Ophthalmology, Harvard Medical School, Boston, MA, USA). American Journal of Pathology, 163(1), 57-68 (English) 2003. CODEN: AJPAA4. ISSN: 0002-9440. Publisher: American Society for Investigative Pathology.

AB Vascular endothelial growth factor-3 (VEGFR-3) plays a critical role in embryonic cardiovascular development and is thought to be expressed exclusively on the lymphatic endothelium, high endothelial venules, and rarely on adult vascular endothelium. Recent evidence also suggests expression of VEGFR-3 on some tumor-associated macrophages. We have studied the expression of VEGFR-3, its **ligand** VEGF-C and the co-receptor neuropilin-2, in normal and inflamed corneas and characterized the phenotype and distribution of VEGFR-3+ cells. Our data demonstrate, for the first time, the expression of VEGFR-3 on corneal dendritic cells (DC) and its up-regulation in inflammation. VEGFR-3+ DC are CD11c+CD45+CD11b+, and are mostly major histocompatibility (MHC) class II-CD80-CD86-, indicating immature DC of a monocytic lineage. During inflammation, there is rapid increase in the number of VEGFR-3+ DC in the cornea associated with heightened membranous expression as compared to a mostly intracellular expression in uninfamed tissue. VEGFR-3+ DC in normal corneas are VEGF-C-neuropilin-2-, but express VEGF-C in inflammation. Interestingly, similar cells are absent both in the normal and inflamed skin. These data demonstrate, for the first time, the expression of VEGFR-3 and VEGF-C on tissue DC, which implicate a novel potential relationship between lymphangiogenesis and leukocyte tracking in the eye.

L6 ANSWER 19 OF 28 CAPLUS COPYRIGHT 2006 ACS on STN

2004:94459 Document No. 140:268080 VEGFR-3 **ligands** and lymphangiogenesis. Jeltsch, Markku Michael (Helsingin Yliopisto, Helsinki, Finland). 95 pp. Avail. From degree-granting institution From: Diss. Abstr. Int., C 2003, 64(2), 359 (English) 2002.

AB Unavailable

L6 ANSWER 20 OF 28 CAPLUS COPYRIGHT 2006 ACS on STN

2002:555522 Document No. 137:119669 VEGFR-3 inhibitor materials and methods. Alitalo, Kari; Koivunen, Erkki; Kubo, Hajime (Ludwig Institute for Cancer Research, USA; Licentia Ltd.). PCT Int. Appl. WO 2002057299 A2 20020725, 149 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-IB99 20020116. PRIORITY: US 2001-2001/PV262476 20010117.

AB The present invention relates to the diagnostics, evaluation, and therapeutic intervention of disorders mediated by the activity of cell surface receptor VEGFR-3, which activity often is stimulated by VEGFR-3 **ligands** VEGF-C and VEGF-D. More particularly, the present invention identifies novel methods and compns. for the inhibition of VEGF-C/D binding to VEGFR-3. The compns. of the present invention will be useful the inhibition of angiogenesis and lymphangiogenesis. Many uses of such compds., for screening samples, imaging, diagnosis, and therapy, are also provided. For example, in one embodiment, the invention provides an

isolated peptide comprising the formula: X1X2X3X4X5X6X7X8, wherein X1 through X8 are amino acid residues.

L6 ANSWER 21 OF 28 CAPLUS COPYRIGHT 2006 ACS on STN

2002:444386 Document No. 137:19390 VEGF-C polypeptides, polynucleotides and anti-VEGF-C antibodies for diagnosing and treating endothelial or angiogenic diseases. Alitalo, Kari; Joukov, Vladimir (Helsinki University Licensing, Ltd., Finland; Ludwig Institute for Cancer Research). U.S. US 6403088 B1 20020611, 71 pp., Cont.-in-part of U.S. 6,245,530. (English). CODEN: USXXAM. APPLICATION: US 1996-601132 19960214. PRIORITY: US 1995-510133 19950801; US 1996-585895 19960112.

AB The invention discloses VEGF-C, a polypeptide **ligand** for Flt4 receptor tyrosine kinase (VEGFR-3), polynucleotides encoding them, and antisense oligonucleotides for diagnosis, therapy and drug screening use. The invention also provides monoclonal and polyclonal antibodies that are reactive with VEGF-C for diagnostic application to monitor angiogenesis, vascularization, lymphatic vessels and their disease states, wound healing, or certain hematopoietic or leukemia cells, and for blockade or activation of Flt4 receptor. The **ligand** and antibody may be coupled to supermagnetic, paramagnetic, electron dense, echogenic, or radioactive agent for imaging.

L6 ANSWER 22 OF 28 CAPLUS COPYRIGHT 2006 ACS on STN

2002:651672 Document No. 137:367450 Expression of the vascular endothelial growth factor receptor-3 (VEGFR-3) and its **ligand** VEGF-C in human colorectal adenocarcinoma. Witte, Deborah; Thomas, Abraham; Ali, Najeeba; Carlson, Nicole; Younes, Mamoun (Department of Pathology, Baylor College of Medicine and The Methodist Hospital, Houston, TX, 77030, USA). Anticancer Research, 22(3), 1463-1466 (English) 2002. CODEN: ANTRD4. ISSN: 0250-7005. Publisher: International Institute of Anticancer Research.

AB Vascular endothelial growth factors (VEGF) are secreted by many tumor types, and are believed to affect tumor growth by promoting angiogenesis through binding to their receptors present on vascular endothelium. Recently, mRNA for VEGF-C the **ligand** for VEGFR-3, was found to be up-regulated in colorectal adenocarcinoma (CRC). The aim of this work was to determine: 1) the distribution of VEGF-C and VEGFR-3 in CRC, and 2) the biol. significance of such expression. Sections of formalin-fixed and paraffin-embedded tissues from 56 CRC were immunohistochem. stained for VEGF-C and VEGFR-3. The type and percent of pos. cells was recorded. Survival anal. was performed using the Kaplan-Meier method. All CRC were pos. for VEGF-C which was present in the cancer cells themselves, as well as in stromal cells. Normal colon epithelium was usually neg. Only ten (17%) of the 56 CRC completely lacked VEGFR-3 expression. VEGFR-3 immunoreactivity was detected in <25% of the cancer cells in 22 cases and in >25% of the cells in 34 cases. Expression of VEGFR-3 in >25% of the cancer cells was associated with significantly poorer overall survival (p<0.05), but not with lymph node metastasis or depth of tumor invasion. Our results suggest that VEGFs promote cancer growth not only by stimulating angiogenesis, but also by acting on receptors present on the cancer cells themselves.

L6 ANSWER 23 OF 28 MEDLINE on STN DUPLICATE 6

2001238036. PubMed ID: 11306510. Vascular endothelial growth factor-B and vascular endothelial growth factor-C expression in renal cell carcinomas: regulation by the von Hippel-Lindau gene and hypoxia. Gunningham S P; Currie M J; Han C; Turner K; Scott P A; Robinson B A; Harris A L; Fox S B. (Department of Anatomical Pathology, Canterbury Health, Christchurch Hospital, New Zealand.) Cancer research, (2001 Apr 1) Vol. 61, No. 7, pp. 3206-11. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB Angiogenesis is essential for tumor growth and metastasis. It is regulated by numerous angiogenic factors, one of the most important being

vascular endothelial growth factor (VEGF). Recently VEGF-B and VEGF-C, two new VEGF family members, have been identified that bind to the tyrosine kinase receptors flt-1 (VEGFR1), KDR (VEGFR2), and flt-4 (VEGFR3). Although the importance of VEGF-A has been shown in renal carcinomas, the contribution of these new **ligands** in kidney tumors is not clear. We have, therefore, measured the mRNA level of VEGF-B and VEGF-C together with their receptors by RNase protection assay (RPA) in 26 normal kidney samples and 45 renal cell cancers. We observed a significant up-regulation of VEGF-B ($P = 0.002$) but not VEGF-C ($P = 0.3$) in neoplastic kidney compared with normal tissues. In addition, although VEGF receptors were higher in tumors than normal kidney, there was a significant up-regulation of only flt-1 ($P = 0.003$) but not KDR ($P = 0.12$) or flt-4 ($P = 0.09$). There was also a significant correlation between VEGF-C and both of its receptors flt-4 ($P = 0.006$) and KDR ($P = 0.03$) but no association between VEGF-B and its receptor flt-1 ($P = 0.23$). A significant increase was observed in flt-1 ($P < 0.001$), KDR ($P = 0.02$), and flt-4 ($P = 0.01$) but not VEGF-B ($P = 0.82$) or VEGF-C ($P = 0.52$) expression in clear cell compared with chromophil (papillary) carcinomas. No significant association was demonstrated between VEGF-B, VEGF-C, flt-1, KDR, and flt-4 with patient sex, patient age, or tumor size ($P > 0.05$). The effect of von Hippel-Lindau (VHL) gene and hypoxia on VEGF-B and VEGF-C expression in the renal carcinoma cell line 786-0 transfected with wild-type and mutant VHL was determined by growing cells under 21% O₂- and 0.1% O₂. In wild-type VHL cells, whereas VEGF-A was significantly up-regulated under hypoxic compared with normoxic conditions ($P < 0.001$), expression of VEGF-C was reduced ($P < 0.002$). Nevertheless, the repression of VEGF-C was lost in mutant VHL cell lines under hypoxia. In contrast VEGF-B was not regulated by VHL despite clear up-regulation in vivo. These findings strongly support an enhanced role for this pathway in clear cell carcinomas by regulating angiogenesis and/or lymphangiogenesis. The study shows that clear cell tumors are able to up-regulate angiogenic growth factor receptors more efficiently than chromophil (papillary), that clear cell tumors can use pathways independent of VHL to regulate angiogenesis, and that this combined regulation may account for their more aggressive phenotype, which suggests that targeting VEGFR1 (flt-1) may be particularly effective in these tumor types.

L6 ANSWER 24 OF 28 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

2000:939062 The Genuine Article (R) Number: 379TV. Vascular endothelial growth factor receptors in the regulation of angiogenesis and lymphangiogenesis. Karkkainen M J; Petrova T V (Reprint). Univ Helsinki, Haartman Inst, Mol Canc Biol Lab, FIN-00014 Helsinki, Finland (Reprint); Univ Helsinki, Haartman Inst, Ludwig Inst Canc Res, FIN-00014 Helsinki, Finland. ONCOGENE (20 NOV 2000) Vol. 19, No. 49, pp. 5598-5605. ISSN: 0950-9232. Publisher: NATURE PUBLISHING GROUP, HOUNDMILLS, BASINGSTOKE RG21 6XS, HAMPSHIRE, ENGLAND. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB VEGFR-1 (Flt-1), VEGFR-2 (KDR) and VEGFR-3 (Flt4) are endothelial specific receptor tyrosine kinases, regulated by members of the vascular endothelial growth factor family, VEGFRs are indispensable for embryonic vascular development, and are involved in the regulation of many aspects of physiological and pathological angiogenesis. VEGF-C and VEGF-D, as **ligands** for VEGFR-3 are also capable of stimulating lymphangiogenesis and at least VEGF-C can enhance lymphatic metastasis, Recent studies have shown that missense mutations within the **VEGFR3** tyrosine kinase domain are associated with human hereditary lymphedema, suggesting an important role for this receptor in the development of the lymphatic vasculature.

L6 ANSWER 25 OF 28 CAPLUS COPYRIGHT 2006 ACS on STN

2001:119862 Document No. 134:290692 VEGF-C and VEGF-D expression in

neuroendocrine cells and their receptor, VEGFR-3, in fenestrated blood vessels in human tissues. Partanen, Taina A.; Arola, Johanna; Saaristo, Anne; Jussila, Lotta; Ora, Ari; Miettinen, Markku; Stacker, Steven A.; Achen, Marc G.; Alitalo, Kari (Molecular / Cancer Biology Laboratory and Department of Pathology, Haartman Institute, University of Helsinki, Helsinki, 00014, Finland). FASEB Journal, 14(13), 2087-2096 (English) 2000. CODEN: FAJOEC. ISSN: 0892-6638. Publisher: Federation of American Societies for Experimental Biology.

AB Recently, vascular endothelial growth factor receptor 3 (VEGFR-3) has been shown to provide a specific marker for lymphatic endothelia in certain human tissues. In this study, we have investigated the expression of VEGFR-3 and its **ligands** VEGF-C and VEGF-D in fetal and adult tissues. VEGFR-3 was consistently detected in the endothelium of lymphatic vessels such as the thoracic duct, but fenestrated capillaries of several organs including the bone marrow, splenic and hepatic sinusoids, kidney glomeruli and endocrine glands also expressed this receptor. VEGF-C and VEGF-D, which bind both VEGFR-2 and VEGFR-3 were expressed in vascular smooth muscle cells. In addition, intense cytoplasmic staining for VEGF-C was observed in neuroendocrine cells such as the α cells of the islets of Langerhans, prolactin secreting cells of the anterior pituitary, adrenal medullary cells, and dispersed neuroendocrine cells of the gastrointestinal tract. VEGF-D was observed in the innermost zone of the adrenal cortex and in certain dispersed neuroendocrine cells. These results suggest that VEGF-C and VEGF-D have a paracrine function and perhaps a role in peptide release from secretory granules of certain neuroendocrine cells to surrounding capillaries.

L6 ANSWER 26 OF 28 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

2000:190523 The Genuine Article (R) Number: 289UM. VEGFc and **VEGFR3** expression in human thyroid pathologies. Shushanov S; Bronstein M; Adelaide J; Jussila L; Tchipsheva T; Jacquemier J; Stavrovskaya A; Birnbaum D (Reprint); Karamysheva A. INSERM, U119, 27 Bd Lei Roure, F-13009 Marseille, France (Reprint); INSERM, U119, F-13009 Marseille, France; Inst J Paoli I Calmettes, F-13009 Marseille, France; Univ Helsinki, Mol Canc Biol Lab, Helsinki, Finland; Russian Endocrinol Res Ctr, Moscow, Russia; Ctr Canc Res, Inst Carcinogenesis, Moscow, Russia. INTERNATIONAL JOURNAL OF CANCER (1 APR 2000) Vol. 86, No. 1, pp. 47-52. ISSN: 0020-7136. Publisher: WILEY-LISS, DIV JOHN WILEY & SONS INC, 605 THIRD AVE, NEW YORK, NY 10158-0012 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB In vertebrates, vascular endothelial growth factors (VEGFs) and their receptors (VEGFRs) are major determinants of angiogenesis. In adults, the interaction between VEGFc and **VEGFR3** (previously FLT4) is more specifically involved in the biology of lymphatics. Using PCR amplification of reverse-transcribed mRNA, we studied the expression of the **VEGFR3** (including its short and long forms) and VEGFc genes in 38 samples of various human thyroid pathologies. **VEGFR3** mRNA was detected in all samples of adenomas, nodular goiters and focal goitrogenic alterations; in all samples of thyroid tissue from patients with auto-immune diseases; and in some samples of adenocarcinomas. VEGFc mRNA was detected in most samples. We studied expression of the **VEGFR3** and VEGFc proteins in thyroid tumors using appropriate antibodies. Co-expression of **VEGFR3** and VEGFc was observed in most samples. Int. J. Cancer 86:47-52, 2000. (C) 2000 Wiley-Liss, Inc.

L6 ANSWER 27 OF 28 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

1999:83085 The Genuine Article (R) Number: 159HL. Role of tyrosine residues and protein interaction domains of SHC adaptor in VEGF Receptor 3 signaling. Fournier E; Blaikie P; Rosnet O; Margolis B; Birnbaum D (Reprint); Borg J P. INSERM, U119, Mol Oncol Lab, F-13258 Marseille, France (Reprint); Dept Internal Med & Biol Chem, Ann Arbor, MI USA; Howard

Hughes Med Inst, Ann Arbor, MI USA. ONCOGENE (14 JAN 1999) Vol. 18, No. 2, pp. 507-514. ISSN: 0950-9232. Publisher: NATURE PUBLISHING GROUP, HOUNDMILLS, BASINGSTOKE RG21 6XS, HAMPSHIRE, ENGLAND. Language: English. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB The **VEGFR3**/FLT4 receptor, which is involved in vasculogenesis and angiogenesis, binds and phosphorylates SHC proteins on tyrosine residues. SHC contains two phosphotyrosine interaction domains: a PTB (Phosphotyrosine Binding) and a SH2 (Src Homology 2) domain. Previous studies have shown that SHC proteins are phosphorylated on Y239/Y240 and Y313 (Y317 in humans) by tyrosine kinases such as the EGF and IL3 receptors. We have investigated which of the SHC tyrosine residues are targeted by the **VEGFR3**/FLT4 kinase and the role of the SHC PTB and SH2 domains in this process. Our results show that Y239/Y240 and Y313 are simultaneously phosphorylated by the kinase, creating GRB2 binding sites. Mutation of SHC PTB, but not SH2, domain interferes with the SHC phosphorylation by **VEGFR3**/FLT4. Soft agar assay experiments revealed that the **VEGFR3**/FLT4 transforming capacity is increased by the mutation of Y239/Y240 to phenylalanines in SHC, suggesting that these two residues mediate an inhibitory signal for cell growth. Mutation of the two phosphorylation sites increases this effect, suggesting that they have a synergistic role.

L6 ANSWER 28 OF 28 MEDLINE on STN DUPLICATE 7
1998167900. PubMed ID: 9435294. Avian VEGF-C: cloning, embryonic expression pattern and stimulation of the differentiation of VEGFR2-expressing endothelial cell precursors. Eichmann A; Corbel C; Jaffredo T; Breant C; Joukov V; Kumar V; Alitalo K; le Douarin N M. (Institut d'Embryologie Cellulaire et Moléculaire du CNRS et du Collège de France, Nogent-sur-Marne, France.. eichmann@infobiogen.fr) . Development (Cambridge, England), (1998 Feb) Vol. 125, No. 4, pp. 743-52. Journal code: 8701744. ISSN: 0950-1991. Pub. country: ENGLAND: United Kingdom. Language: English.

AB VEGF-C is a recently discovered secreted polypeptide related to the angiogenic mitogen VEGF. We have isolated the quail VEGF-C cDNA and shown that its protein product is secreted from transfected cells and interacts with the avian **VEGFR3** and VEGFR2. In situ hybridization shows that quail VEGF-C mRNA is strongly expressed in regions destined to be rich in lymphatic vessels, particularly the mesenteries, mesocardium and myotome, in the region surrounding the jugular veins, and in the kidney. These expression sites are similar to those observed in the mouse embryo (E. Kukk, A. Lymboussaki, S. Taira, A. Kaipainen, M. Jeltsch, V. Joukov and K. Alitalo, 1996, Development 122, 3829-3837). We have observed **VEGFR3**-positive endothelial cells in proximity to most of the VEGF-C-expressing sites, suggesting functional relationships between this receptor-ligand couple. The comparison of the VEGF and VEGFR2 knockout phenotypes had suggested the existence of another ligand for VEGFR2. We therefore investigated the effect of VEGF-C on VEGFR2-positive cells isolated from the posterior mesoderm of gastrulating embryos. We have recently shown that VEGF binding triggers endothelial differentiation of these cells, whereas hemopoietic differentiation appears to be mediated by binding of a so far unidentified VEGFR2 ligand. We show here that VEGF-C also triggers endothelial differentiation of these cells, presumably via VEGFR2. These results indicate that VEGF and VEGF-C can act in a redundant manner via VEGFR2. In conclusion, VEGF-C appears to act during two different developmental phases, one early in posterior mesodermal VEGFR2-positive endothelial cell precursors which are negative for **VEGFR3** and one later in regions rich in lymphatic vessels at a time when endothelial cells express both VEGFR2 and **VEGFR3**.

=> s VEGF-C

L7 2842 VEGF-C

=> s 17 and antagonist

L8 95 L7 AND ANTAGONIST

=> s 18 and derivative

L9 4 L8 AND DERIVATIVE

=> dup remove 19

PROCESSING COMPLETED FOR L9

L10 4 DUP REMOVE L9 (0 DUPLICATES REMOVED)

=> d 110 1-4 cbib abs

L10 ANSWER 1 OF 4 MEDLINE on STN

2006099908. PubMed ID: 16489074. Nitric oxide in breast cancer: induction of vascular endothelial growth factor-C and correlation with metastasis and poor prognosis. Nakamura Yasushi; Yasuoka Hironao; Tsujimoto Masahiko; Yoshidome Katsuhide; Nakahara Masaaki; Nakao Kazuyasu; Nakamura Misa; Kakudo Kennichi. (Department of Pathology, Wakayama Medical University, Wakayama City, Wakayama, Japan.. nakamur@wakayama-med.ac.jp) . Clinical cancer research : an official journal of the American Association for Cancer Research, (2006 Feb 15) Vol. 12, No. 4, pp. 1201-7. Journal code: 9502500. ISSN: 1078-0432. Pub. country: United States. Language: English.

AB PURPOSE: Metastasis to regional lymph nodes through the lymphatic vessels is a common step in the progression of cancer. Recent evidence suggests that tumor production of vascular endothelial growth factor-C (**VEGF-C**) promotes lymphangiogenesis, which in turn promotes lymphatic metastasis. Nitric oxide (NO) may also increase metastatic ability in human cancers. EXPERIMENTAL DESIGN: Nitrite/nitrate levels and **VEGF-C** production were assessed in MDA-MB-231 breast cancer cells after induction and/or inhibition of NO synthesis. Formation of nitrotyrosine, a biomarker for peroxynitrate formation from NO in vivo, was analyzed in primary human breast carcinoma with long-term follow-up. The relationship between nitrotyrosine levels and lymph node status, **VEGF-C** immunoreactivity, and other established clinicopathologic variables, as well as prognosis, was analyzed. RESULTS: Production of nitrite/nitrate and **VEGF-C** in MDA-MB-231 cells was increased by treatment with the NO donor DETA NONOate. The NO synthase inhibitor N(G)-nitro-L-arginine methyl ester eliminated this increase. High-grade nitrotyrosine staining was observed in 57.5% (65 of 113) of the invasive breast carcinomas. Nitrotyrosine levels were significantly correlated with **VEGF-C** immunoreactivity and lymph node metastasis. Survival curves determined by the Kaplan-Meier method showed that high nitrotyrosine levels were associated with reduced disease-free and overall survival. In multivariate analysis, high nitrotyrosine levels emerged as a significant independent predictor for overall survival. CONCLUSIONS: Our data showed a role for NO in stimulating **VEGF-C** expression in vitro. Formation of its biomarker nitrotyrosine was also correlated with **VEGF-C** expression and lymph node metastasis. Furthermore, high nitrotyrosine levels may serve as a significant prognostic factor for long-term survival in breast cancer.

L10 ANSWER 2 OF 4 MEDLINE on STN

2006030913. PubMed ID: 16278821. Inducible nitric oxide synthase activity correlates with lymphangiogenesis and vascular endothelial growth factor-C expression in head and neck squamous cell carcinoma. Franchi Alessandro; Massi Daniela; Santucci Marco; Masini Emanuela; Degl'Innocenti Duccio Rossi; Magnelli Lucia; Fanti Elena; Naldini Antonella; Ardinghi Camilla; Carraro Fabio; Gallo Oreste. (Department of Human Pathology and Oncology, University of Florence, Italy.. Franchi@unifi.it) . The Journal of pathology, (2006 Feb) Vol. 208, No. 3, pp. 439-45. Journal code: 0204634. ISSN: 0022-3417. Pub. country: England: United Kingdom. Language: English.

AB Nitric oxide (NO) is a diatomic free radical molecule that has been implicated in tumour angiogenesis and progression of head and neck squamous cell carcinoma (HNSCC). However, the mechanism underlying the effect of NO on tumour spread remains largely unknown. Tumour lymphangiogenesis has recently received considerable attention and there is increasing evidence that it is relevant for metastasis to lymph nodes in HNSCC. Here, we study the correlation between inducible NOS synthase (iNOS) activity and lymphangiogenesis in a series of 60 HNSCCs and the possible involvement of the lymphangiogenic factor vascular endothelial growth factor (VEGF)-C. HNSCC presenting with lymph node metastasis had a significantly higher lymphatic vessel density in both the tumour mass and the peritumour area ($p = 0.006$ and $p = 0.001$, respectively). Similarly, tumours with lymph node metastasis showed greater lymphatic vessel area than tumours with no lymph node involvement ($p = 0.001$ for intratumour lymphatics and $p < 0.001$ for peritumour lymphatics). iNOS activity measured in specimens from the tumour periphery correlated strongly with both lymphatic vessel density and lymphatic vessel area ($p = 0.01$, $r_s = 0.45$ and $p < 0.001$, $r_s = 0.725$, respectively). Conversely, these correlations were not observed in specimens from the tumour core. In addition, VEGF-C mRNA expression was significantly elevated in tumours with high iNOS activity ($p = 0.008$, $r_s = 0.563$), and VEGF-C expression correlated positively with the presence of lymph node metastases ($p = 0.03$). In vitro, in the A431 human squamous carcinoma cell line, exogenous and endogenous stimulation of the iNOS pathway led to up-regulation of VEGF-C, which was blocked by the NOS inhibitor L-NNA. Taken together, our results indicate that iNOS activity may promote lymphangiogenesis and spread to lymph nodes in HNSCC, with the possible involvement of VEGF-C.

L10 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2006 ACS on STN
 2005:1242960 Document No. 144:11543 Enhancement of **antagonist** activity of aptamers by conjugation to high molecular weight materials. Calias, Pericles; Cook, Gary P.; Shima, David T.; Adamis, Anthony P.; Ng, Yin-Shan; Robinson, Gregory S.; Turner, David I.; Ganley, Mary A. (Eyetech Pharmaceuticals, Inc., USA). PCT Int. Appl. WO 2005110489 A2 20051124, 91 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IS, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2005-US12469 20050413. PRIORITY: US 2004-2004/PV56160U 20040413; US 2005-2005/PV658819 20050304.

AB The invention provides compns. and methods for making and using sterically enhanced aptamer **antagonist** conjugates which include a nucleic acid sequence having a specific affinity for a target mol. and a soluble, high mol. weight steric group that augments or facilitates the **antagonist** activity of the aptamer. inhibition of binding to, or interaction with, the target mol. binding partner by the target mol. when bound to the aptamer conjugate. The present invention also provides methods and formulations for ocular delivery of a biol. active mol. by attaching a charged moiety to the biol. active mol. and delivering the biol. active mol. by iontophoresis. Iontophoresis of a biol. active mol. that is conjugated to a high mol. weight neutral moiety, is enhanced by replacing the high mol. weight neutral moiety with a charged mol. of comparable size. Thus, the effect on VEGFR-1 (Flt-1) **antagonist** activity of conjugation of chemical modified, anti-VEGF oligoribonucleotide to various high mol. weight materials, i.e., PEG, dextran, and CMC, was studied. Also examined was the effects of mol. weight and hydrodynamic volume

of

the high mol. weight material on **antagonist** activity.

L10 ANSWER 4 OF 4 MEDLINE on STN

2005458522. PubMed ID: 16102752. Modulating furin activity with designed mini-PDX peptides: synthesis and in vitro kinetic evaluation. Basak Ajoy; Lotfipour Farzaneh. (Diseases of Aging Program, Regional Protein Chemistry Center, Ottawa Health Research Institute, University of Ottawa, Loeb Building, 725 Parkdale Ave, Ottawa, ON, K1Y 4E9, Canada.. abasak@ohri.ca) . FEBS letters, (2005 Aug 29) Vol. 579, No. 21, pp. 4813-21. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB A peptide was designed from reactive site loop structure of alpha1 Antitrypsin Portland known as alpha1 PDX as a novel mini-PDX inhibitor of furin. The sequence was derived from (367-394) that contains the crucial furin cleavage motif RIPR382. A P3 mutant replacing Ile380 by Leu was prepared as a first model peptide. A Cys residue was inserted at each terminal of the peptide for purpose of cyclisation which was accomplished by air or iodine-induced oxidation. This mini-PDX peptide both cyclic and acyclic form inhibited in vitro furin activity (IC50 in nM) when measured against either substrates Boc-RVRRdown double arrow MCA or QVEGF-C [Abz-QVHSIIRRdown double arrow SLP-Y(NO2)-A-CONH2, Abz=2-amino benzoic acid and Y(NO2)=3-nitro tyrosine], latter being derived from vascular endothelial growth factor-C (**VEGF-C**) processing site. The geometrically constrained structure mimicking PDX reactive loop is crucial for enzyme inhibition. Our study further revealed that both mini-PDX peptides inactivate furin in a slow tight binding manner, with disulfide-bridged cyclic form being slightly more potent. Unlike PDX, these peptides inhibit furin via a different mechanistic pathway. The study provides an alternate strategy for development of efficient peptide-based inhibitors of Proprotein Convertases including furin.

=> s l7 and derivative

L11 28 L7 AND DERIVATIVE

=> dup remove l11

PROCESSING COMPLETED FOR L11

L12 20 DUP REMOVE L11 (8 DUPLICATES REMOVED)

=> d l12 1-20 cbib abs

L12 ANSWER 1 OF 20 MEDLINE on STN

2006099908. PubMed ID: 16489074. Nitric oxide in breast cancer: induction of vascular endothelial growth factor-C and correlation with metastasis and poor prognosis. Nakamura Yasushi; Yasuoka Hironao; Tsujimoto Masahiko; Yoshidome Katsuhide; Nakahara Masaaki; Nakao Kazuyasu; Nakamura Misa; Kakudo Kennichi. (Department of Pathology, Wakayama Medical University, Wakayama City, Wakayama, Japan.. nakamur@wakayama-med.ac.jp) . Clinical cancer research : an official journal of the American Association for Cancer Research, (2006 Feb 15) Vol. 12, No. 4, pp. 1201-7. Journal code: 9502500. ISSN: 1078-0432. Pub. country: United States. Language: English.

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L12 ANSWER 2 OF 20 MEDLINE on STN

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L12 ANSWER 3 OF 20 CAPLUS COPYRIGHT 2006 ACS on STN

2005:1242960 Document No. 144:11543 Enhancement of antagonist activity of aptamers by conjugation to high molecular weight materials. Calias, Pericles; Cook, Gary P.; Shima, David T.; Adamis, Anthony P.; Ng, Yin-Shan; Robinson, Gregory S.; Turner, David I.; Ganley, Mary A. (Eyetechn

Pharmaceuticals, Inc., USA). PCT Int. Appl. WO 2005110489 A2 20051124, 91 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IS, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2005-US12469 20050413. PRIORITY: US 2004-2004/PV56160U 20040413; US 2005-2005/PV658819 20050304.

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weight neutral moiety with a charged mol. of comparable size. Thus, the effect on VEGFR-1 (Flt-1) antagonist activity of conjugation of chemical modified, anti-VEGF oligoribonucleotide to various high mol. weight materials, i.e., PEG, dextran, and CMC, was studied. Also examined was the effects of mol. weight and hydrodynamic volume of the high mol. weight material on antagonist activity.

L12 ANSWER 4 OF 20 CAPLUS COPYRIGHT 2006 ACS on STN
2005:122784 Document No. 142:212747 Fusion proteins of vascular endothelial growth factor homology domains and heparin-binding domains for use as ligands of vascular endothelial growth factor receptor 3. Alitalo, Kari; He, Yulong; Tammela, Toumas (Finland). U.S. Pat. Appl. Publ. US 2005032697 A1 20050210, 119 pp., Cont.-in-part of U.S. Ser. No. 669,176. (English). CODEN: USXXCO. APPLICATION: US 2004-868577 20040614. PRIORITY: US 2003-2003/PV47811U 20030612; US 2003-2003/PV47839U 20030612; US 2003-2003/669176 20030923.

AB Synthetic ligands for vascular endothelial growth factor receptor 3 that are fusion proteins of a heparin-binding domain and a vascular endothelial growth factor homol. domain and that show greater heparin-binding are described for use in therapeutic control of angiogenesis. These synthetic ligands show greater binding to heparin and the VEGFR3 receptor than its native ligands, **VEGF-C** and **VEGF-D**. A fusion protein of **VEGF-C** and the heparin-binding domain of vascular endothelial growth factor was manufactured by expression of the corresponding gene in 293T or 293EBNA cells. The proteins had greater lymphangiogenic activity than **VEGF-C** in guinea pigs, although the induced blood vessels were leaky.

L12 ANSWER 5 OF 20 MEDLINE on STN
2005458522. PubMed ID: 16102752. Modulating furin activity with designed mini-PDX peptides: synthesis and in vitro kinetic evaluation. Basak Ajoy; Lotfipour Farzaneh. (Diseases of Aging Program, Regional Protein Chemistry Center, Ottawa Health Research Institute, University of Ottawa, Loeb Building, 725 Parkdale Ave, Ottawa, ON, K1Y 4E9, Canada.. abasak@ohri.ca) . FEBS letters, (2005 Aug 29) Vol. 579, No. 21, pp. 4813-21. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

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furin cleavage motif R1PR382. A P3 mutant replacing Ile380 by Leu was prepared as a first model peptide. A Cys residue was inserted at each terminal of the peptide for purpose of cyclisation which was accomplished by air or iodine-induced oxidation. This mini-PDX peptide both cyclic and acyclic form inhibited in vitro furin activity (IC50 in nM) when measured against either substrates Boc-RVRRdown double arrow MCA or QVEGF-C [Abz-QVHSIIRdown double arrow SLP-Y(NO2)-A-CONH2, Abz=2-amino benzoic acid and Y(NO2)=3-nitro tyrosine], latter being derived from vascular endothelial growth factor-C (**VEGF-C**) processing site. The geometrically constrained structure mimicking PDX reactive loop is crucial for enzyme inhibition. Our study further revealed that both mini-PDX peptides inactivate furin in a slow tight binding manner, with disulfide-bridged cyclic form being slightly more potent. Unlike PDX, these peptides inhibit furin via a different mechanistic pathway. The study provides an alternate strategy for development of efficient peptide-based inhibitors of Proprotein Convertases including furin.

L12 ANSWER 6 OF 20 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN

2005413576 EMBASE A genetic *Xenopus laevis* tadpole model to study lymphangiogenesis. Ny A.; Koch M.; Schneider M.; Neven E.; Tong R.T.; Maity S.; Fischer C.; Plaisance S.; Lambrechts D.; Heligon C.; Terclavers S.; Ciesiolka M.; Kalin R.; Wing Y.M.; Senn I.; Wyns S.; Lupu F.; Brandli A.; Vleminckx K.; Collen D.; Dewerchin M.; Conway E.M.; Moons L.; Jain R.K.; Carmeliet P.. P. Carmeliet, Flanders Interuniversity Institute for Biotechnology, Center for Transgene Technology and Gene Therapy, KULEuven, Herestraat 49, Leuven, B-3000, Belgium. peter.carmeliet@med.kuleuven.be. Nature Medicine Vol. 11, No. 9, pp. 998-1004 2005. Refs: 28.

ISSN: 1078-8956. CODEN: NAMEFI

Pub. Country: United Kingdom. Language: English. Summary Language: English.

Entered STN: 20051103. Last Updated on STN: 20051103

AB Lymph vessels control fluid homeostasis, immunity and metastasis. Unraveling the molecular basis of lymphangiogenesis has been hampered by the lack of a small animal model that can be genetically manipulated. Here, we show that *Xenopus* tadpoles develop lymph vessels from lymphangioblasts or, through transdifferentiation, from venous endothelial cells. Lymphangiography showed that these lymph vessels drain lymph, through the lymph heart, to the venous circulation. Morpholino-mediated knockdown of the lymphangiogenic factor Prox1 caused lymph vessel defects and lymphedema by impairing lymphatic commitment. Knockdown of vascular endothelial growth factor C (**VEGF-C**) also induced lymph vessel defects and lymphedema, but primarily by affecting migration of lymphatic endothelial cells. Knockdown of **VEGF-C** also resulted in aberrant blood vessel formation in tadpoles. This tadpole model offers opportunities for the discovery of new regulators of lymphangiogenesis.

L12 ANSWER 7 OF 20 CAPLUS COPYRIGHT 2006 ACS on STN

2004:249186 Document No. 140:264484 Screening for inhibitors of vascular endothelial growth factor receptor 3 for use as inhibitors of metastasis or tumor-induced lymphangiogenesis. Krishnan, Jaya; Sleemann, Jonathan (Forschungszentrum Karlsruhe GmbH, Germany). Ger. Offen. DE 10242663 A1 20040325, 11 pp. (German). CODEN: GWXXBX. APPLICATION: DE 2002-10242663 20020913.

AB A method of identifying inhibitors of tumor metastasis formation or tumor-induced lymphangiogenesis that specifically inhibit activation in vivo of the lymphangiogenesis regulating cell receptor vascular endothelial growth factor receptor VEGFR-3 is described. The method involves using cells expressing a gene for the vascular endothelial growth factor C **derivative** ΔNACVEGF-C/Cys152Ser. This variant can induced angiogenesis and metastasis in weakly metastasizing cell lines and is

specific for VEGFR-3. Testing can be carried out in immune-competent animal hosts.

L12 ANSWER 8 OF 20 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN

2004135971 EMBASE Development of vascular endothelial growth factor receptor (VEGFR) kinase inhibitors as anti-angiogenic agents in cancer therapy. Underiner T.L.; Ruggeri B.; Gingrich D.E.. T.L. Underiner, Cephalon Inc., 145 Brandywine Parkway, West Chester, PA 19380, United States. tunderin@cephalon.com. Current Medicinal Chemistry Vol. 11, No. 6, pp. 731-745 2004.

Refs: 115.

ISSN: 0929-8673. CODEN: CMCHE7

Pub. Country: Netherlands. Language: English. Summary Language: English.

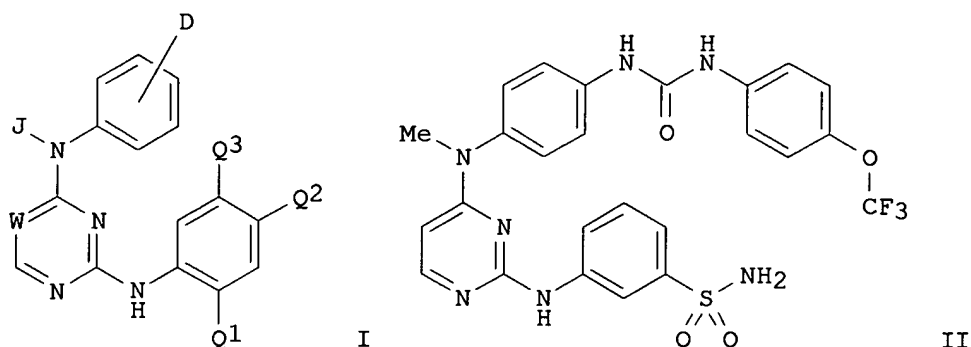
Entered STN: 20040412. Last Updated on STN: 20040412

AB Among the known angiogenic growth factors and cytokines implicated in the modulation of normal and pathological angiogenesis, the VEGF family (VEGF-A, VEGF-B, **VEGF-C**, VEGF-D) and their corresponding receptor tyrosine kinases [VEGFR-1 (Flt-1), VEGFR-2 (Flk-1, KDR), and VEGFR-3 (Flt-4)] play a paramount and indispensable role in regulating the multiple facets of the angiogenic and lymphangiogenic processes, as well as the induction of vascular permeability and inflammation. The receptor VEGFR-2/KDR is the principal one through which VEGFs exert their mitogenic, chemotactic, and vascular permeabilizing effects on the host vasculature. Increased expression of VEGFs by tumor cells and VEGFR-2/KDR and VEGFR-1/Flt-1 by the tumor-associated vasculature are a hallmark of a variety of human and rodent tumors in vivo and correlates with tumor growth rate, micro-vessel density/proliferation, tumor metastatic potential, and poorer patient prognosis in a variety of malignancies. Approaches to disrupting the VEGF/VEGFR signaling cascade range from biological agents (soluble receptors, anti-VEGF and anti-VEGFR-2 antibodies, and VEGF transcription inhibitors) to small molecule ATP competitive VEGFR inhibitors. Examples from this latter class that are currently in clinical development include compounds from distinct chemical classes such as: indolin-2-ones, anilinoquinazolines, anilinophthalazines, isothiazoles, indolo- and indenocarbazoles. The structure activity relationships, biochemical and pharmacological profile of optimized representatives from each of these classes constitute the subject matter of this review. .COPYRG. 2004 Bentham Science Publishers Ltd.

L12 ANSWER 9 OF 20 CAPLUS COPYRIGHT 2006 ACS on STN

2003:633670 Document No. 139:180077 Preparation of (phenylamino)pyrimidine **derivatives** as TIE-2 and/or VEGFR-2 inhibitors for treatment of hyperproliferative diseases. Cheung, Mui; Nailor, Kristen Elizabeth; Sammond, Douglas Mccord; Veal, James Marvin (Smithkline Beecham Corporation, USA). PCT Int. Appl. WO 2003066601 A1 20030814, 106 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US3816 20030207. PRIORITY: US 2002-2002/PV355046 20020208.

GI



AB The title pyrimidine **derivs.** with general formula of I [wherein W = N or CR; R = H, halo, or CN; J = H, alkyl, haloalkyl, aralkyl, cyanoalkyl, (CH₂)_pC=CH(CH₂)_qH, (CH₂)_pC.tplbond.C(CH₂)_qH, or cycloalkyl; p = 1-3; q = 0-1; D = (un)substituted amino; Q1 = H, halo, haloalkyl, alkyl, alkoxy, or haloalkoxy; Q2 = A1 or A2; Q3 = A1 when Q2 = A2 or A2 when Q2 = A1; A1 = H, halo, alkyl, haloalkyl, or (un)substituted OH; A2 = (Z)m-(Z1)-(Z2); Z = CH₂ where m = 0-3, (un)substituted amino where m = 0-1, O where m = 0-1, or (un)substituted CH₂-amino where m = 0-1; Z1 = SO₂, SO, or CO; Z2 = alkyl, cycloalkyl, heterocyclyl, aryl, arylamino, aralkyl, aralkoxy, heteroaryl, or (un)substituted amino] and salts, solvates, and phys. functional **derivs.** thereof, which are useful as tyrosine kinase TIE-2 and/or VEGFR-2 inhibitors for the treatment of hyperproliferative diseases, are prepared For example, 3-[[4-[(4-aminophenyl)(methyl)amino]pyrimidin-2-yl]amino]benzenesulfonamide (preparation given) was reacted with (4-trifluoromethoxy)phenyl isocyanate in MeCOME to give II. Some of compds. I showed "-log(IC₅₀)" of >7.0 against human TIE2-FP, VEGF-E, and **VEGF-C**.

L12 ANSWER 10 OF 20 CAPLUS COPYRIGHT 2006 ACS on STN

2003:293770 Document No. 139:240734 Vascular endothelial growth factor (VEGF) receptor-2 signaling mediates **VEGF-C**

ΔNAC- and VEGF-A-induced angiogenesis in vitro. Tille, Jean-Christophe; Wang, Xueyan; Lipson, Kenneth E.; McMahon, Gerald; Ferrara, Napoleone; Zhu, Zhenping; Hicklin, Daniel J.; Sleeman, Jonathan P.; Eriksson, Ulf; Alitalo, Kari; Pepper, Michael S. (Department of Cell Biology and Morphology, University Medical Center, Geneva, Switz.). Experimental Cell Research, 285(2), 286-298 (English) 2003. CODEN: ECREAL. ISSN: 0014-4827. Publisher: Elsevier Science.

AB Angiogenesis and lymphangiogenesis are regulated by members of the vascular endothelial growth factor (VEGF) family of cytokines, which mediate their effects via tyrosine kinase VEGF receptors -1, -2, and -3. The authors have used wild-type and mutant forms of VEGFs -A, -B, and -C, a pan-VEGFR tyrosine kinase inhibitor (SU5416) as well as neutralizing anti-VEGFR-2 antibodies, to determine which VEGF receptor(s) are required for bovine endothelial cell invasion and tube formation in vitro. This was compared to the ability of these cytokines to induce expression of members of the plasminogen activator (PA)-plasmin system. The authors found that cytokines which bind VEGFR-2 (human VEGF-A, human VFM23A, human **VEGF-C**.DELTA.NAC, and rat VEGF-C152) induced invasion, tube formation, urokinase-type-PA, tissue-type-PA, and PA inhibitor-1, invasion and tube formation as well as signaling via the MAP kinase pathway were efficiently blocked by SU5416 and anti-VEGFR-2 antibodies. In contrast, cytokines and mutants which exclusively bind VEGFR-1 (human VFM17 and human VEGF-B) had no effect on invasion and tube formation or on the regulation of gene expression. The authors were unable to identify cytokines which selectively stimulate bovine VEGFR-3 in the authors' system. Taken together, these findings point to the central

role of VEGFR-2 in the angiogenic signaling pathways induced by
VEGF-C.DELTA.NAC and VEGF-A.

L12 ANSWER 11 OF 20 CAPLUS COPYRIGHT 2006 ACS on STN

2002:574945 Document No. 137:135506 Methods and compositions for promoting angiogenesis by delivering angiogenic factors. Pawliuk, Robert; Leboulch, Philippe (Genetix Pharmaceuticals, Inc., USA). PCT Int. Appl. WO 2002058718 A2 20020801, 38 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US1666 20020118. PRIORITY: US 2001-2001/PV264457 20010126.

AB The present invention provides novel methods and compns. for promoting angiogenesis to treat a variety of tissue ischemias, including peripheral and myocardial ischemia. Selected angiogenic factors or synergistic combinations of factors, functional analogs of such factors or combinations of factors, or nucleic acids encoding such factors or combinations of factors, are delivered to a localized area of tissue in an amount effective to induce angiogenesis within the area of tissue. The invention further includes improved methods and vehicles for delivering such factors.

L12 ANSWER 12 OF 20 CAPLUS COPYRIGHT 2006 ACS on STN

2002:555522 Document No. 137:119669 VEGFR-3 inhibitor materials and methods. Alitalo, Kari; Koivunen, Erkki; Kubo, Hajime (Ludwig Institute for Cancer Research, USA; Licentia Ltd.). PCT Int. Appl. WO 2002057299 A2 20020725, 149 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-IB99 20020116. PRIORITY: US 2001-2001/PV262476 20010117.

AB The present invention relates to the diagnostics, evaluation, and therapeutic intervention of disorders mediated by the activity of cell surface receptor VEGFR-3, which activity often is stimulated by VEGFR-3 ligands **VEGF-C** and VEGF-D. More particularly, the present invention identifies novel methods and compns. for the inhibition of **VEGF-C/D** binding to VEGFR-3. The compns. of the present invention will be useful the inhibition of angiogenesis and lymphangiogenesis. Many uses of such compds., for screening samples, imaging, diagnosis, and therapy, are also provided. For example, in one embodiment, the invention provides an isolated peptide comprising the formula: X1X2X3X4X5X6X7X8, wherein X1 through X8 are amino acid residues.

L12 ANSWER 13 OF 20 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN

2002440932 EMBASE Lymphatic endothelial regulation, lymphoedema, and lymph node metastasis. Karkkainen M.J.; Alitalo K.. M.J. Karkkainen, Molecular/Cancer Biology Laboratory, Helsinki University Hospital, University of Helsinki, PO Box 63 (Haartmaninkatu 8), 00014 Helsinki, Finland. Marika.Karkkainen@Helsinki.Fi. Seminars in Cell and Developmental Biology Vol. 13, No. 1, pp. 9-18 2002.
Refs: 100.

ISSN: 1084-9521. CODEN: SCDBFX

Pub. Country: United Kingdom. Language: English. Summary Language: English.

Entered STN: 20021219. Last Updated on STN: 20021219

AB Vascular endothelial growth factor receptor-3 (VEGFR-3) mediates lymphatic endothelial cell (LEC) growth, migration, and survival by binding **VEGF-C** and VEGF-D. Recent studies have revealed new regulators of the lymphatic endothelium, such as the transcription factor Prox1, and the cell surface proteins podoplanin and lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1). Furthermore, the isolation of LECs now allows detailed molecular studies of the factors regulating the lymphatic vasculature. These studies are aimed at targeting the lymphatic vasculature in the treatment of various diseases, such as tumour metastasis and lymphoedema.

L12 ANSWER 14 OF 20 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

2001:945005 The Genuine Article (R) Number: 494QQ. Multiple forms of mouse vascular endothelial growth factor-D are generated by RNA splicing and proteolysis. Baldwin M E; Roufail S; Halford M M; Alitalo K; Stacker S A; Achen M G (Reprint). Royal Melbourne Hosp, Ludwig Inst Canc Res, POB 2008, Melbourne, Vic 3050, Australia (Reprint); Royal Melbourne Hosp, Ludwig Inst Canc Res, Melbourne, Vic 3050, Australia; Univ Helsinki, Mol Canc Biol Lab, FIN-00014 Helsinki, Finland. JOURNAL OF BIOLOGICAL CHEMISTRY (23 NOV 2001) Vol. 276, No. 47, pp. 44307-44314. ISSN: 0021-9258. Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The secreted glycoprotein vascular endothelial growth factor-D (VEGF-D) is angiogenic, lymphangiogenic, and promotes metastatic spread of tumor cells via lymphatic vessels. VEGF-D consists of a receptor-binding domain (VEGF homology domain) and N- and G terminal propeptides. Proteolytic processing produces numerous forms of human VEGF-D, including fully processed **derivatives** (containing only the VEGF homology domain), partially processed, and unprocessed **derivatives**. Proteolysis is essential to generate human VEGF-D that binds the angiogenic receptor VEGF receptor-2 (VEGFR-2) and the lymphangiogenic receptor VEGFR-3 with high affinity. Here, we report that alternative use of an RNA splice donor site in exon 6 of the mouse VEGF-D gene produces two different protein isoforms, VEGF-D-358 and VEGF-D-326, with distinct C termini. The two isoforms were both expressed in all adult mouse tissues and embryonic stages of development analyzed. Both isoforms are proteolytically processed in a similar fashion to human VEGF-D to generate a range of secreted **derivatives** and bind and cross-link VEGFR-3 with similar potency. The isoforms are differently glycosylated when expressed in vitro. This study demonstrates that RNA splicing, protein glycosylation, and proteolysis are mechanisms for generating structural diversity of mouse VEGF-D.

L12 ANSWER 15 OF 20 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN

2002082702 EMBASE Characterization of indolinones which preferentially inhibit **VEGF-C** and VEGF-D-induced activation of VEGFR-3 rather than VEGFR-2. Kirkin V.; Mazitschek R.; Krishnan J.; Steffen A.; Waltenberger J.; Pepper M.S.; Giannis A.; Sleeman J.P.. J.P. Sleeman, Forschungszentrum Karlsruhe, Institute of Genetics, P.O. Box 3640, D-76021 Karlsruhe, Germany. sleeman@itg.fzk.de. European Journal of Biochemistry Vol. 268, No. 21, pp. 5530-5540 2001. Refs: 43.

ISSN: 0014-2956. CODEN: EJBCAI

Pub. Country: United Kingdom. Language: English. Summary Language: English.

Entered STN: 20020314. Last Updated on STN: 20020314

AB **VEGF-C** and VEGF-D are lymphangiogenic factors that bind to and activate VEGFR-3, a *fms*-like tyrosine kinase receptor whose expression is limited almost exclusively to lymphatic endothelium in the

adult. Processed forms of **VEGF-C** and VEGF-D can also activate VEGFR-2, a key player in the regulation of angiogenesis. There is increasing evidence to show that these receptor-ligand interactions play a pivotal role in a number of pathological situations. Inhibition of receptor activation by **VEGF-C** and VEGF-D could therefore be pharmaceutically useful. Furthermore, to understand the different roles of **VEGF-C**, VEGF-D, VEGFR-2 and VEGFR-3 in pathological situations it will be necessary to dissect the complex interactions of these ligands and their receptors. To facilitate such studies we cloned, sequenced and characterized the expression of rat **VEGF-C** and VEGF-D. We showed that Cys152→Ser mutants of processed rat **VEGF-C** can activate VEGFR-3 but not VEGFR-2, while the corresponding mutation in rat VEGF-D inhibits its ability to activate both VEGFR-2 and VEGFR-3. We also synthesized and characterized indolinones that differentially block **VEGF-C**- and VEGF-D-induced VEGFR-3 kinase activity compared to that of VEGFR-2. These tools should be useful in analysing the different activities and roles of **VEGF-C**, VEGF-D and their ligands, and in blocking VEGFR-3-mediated lymphangiogenesis.

L12 ANSWER 16 OF 20 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN

2001149086 EMBASE Lymphangiogenesis and the vascular endothelial growth factor receptor (VEGFR)-3 in gastric cancer. Yonemura Y.; Fushida S.; Bando E.; Kinoshita K.; Miwa K.; Endo Y.; Sugiyama K.; Partanen T.; Yamamoto H.; Sasaki T.. Y. Yonemura, Second Department of Surgery, School of Medicine, Kanazawa University, Takara-Machi 13-1, Kanazawa 920, Japan. yonemu@med.kanazawa-u.ac.jp. European Journal of Cancer Vol. 37, No. 7, pp. 918-923 2001.
Refs: 22.

ISSN: 0959-8049. CODEN: EJCAEL

S 0959-8049(01)00015-6. Pub. Country: United Kingdom. Language: English. Summary Language: English.

Entered STN: 20010503. Last Updated on STN: 20010503

AB Vascular endothelial growth factor C (**VEGF-C**) is the only factor known to cause lymphangiogenesis. We studied the correlation between **VEGF-C** and vascular endothelial growth factor receptor-3 (VEGFR-3) expression of 85 primary gastric cancers by reverse transcriptase-polymerase chain reaction (RT-PCR) and immunohistochemistry, and the results were correlated with the number of lymphatic vessels, stained with anti-VEGFR-3 antibody. RT-PCR and immunohistology demonstrated that **VEGF-C** was mainly produced from cancer cells, but not from stromal elements. Morphologically, VEGFR-3 expression was detected in the endothelial cells of the stromal lymphatic vessels. There was a statistically positive correlation between the incidence of **VEGF-C** and VEGFR-3 mRNA expression in the primary tumours (P = 0.0002). The number of VEGFR-3-positive lymphatic vessels in **VEGF-C** mRNA positive tumours was significantly larger than that in **VEGF-C**-negative tumours. The number of VEGFR-3-positive vessels in the tumour stroma was closely related to the grade of lymphatic invasion of gastric cancer. These results strongly indicate that **VEGF-C** may induce the proliferation of lymphatic vessels in the stroma of primary gastric cancer via activation of VEGFR-3, expressed on the endothelial cells of lymphatic vessels. In these circumstances, cancer cells can easily invade the lymphatic vessel, because of the increase of the contact points of cancer cells with the lymphatic vessels. .COPYRGHT. 2001 Elsevier Science Ltd.

L12 ANSWER 17 OF 20 MEDLINE on STN

2001446511. PubMed ID: 11494038. Targeting of doxorubicin to ES-2 human ovarian cancers in nude mice by linking to an analog of luteinizing hormone-releasing hormone improves its effectiveness. Arencibia J M;

Schally A V; Krupa M; Bajo A M; Nagy A; Szepeshazi K; Plonowski A.
(Endocrine, Polypeptide and Cancer Institute, Veterans Affairs Medical
Center, 1601 Perdido Street, New Orleans, LA 70112-1262, USA.)
International journal of oncology, (2001 Sep) Vol. 19, No. 3, pp. 571-7.
Journal code: 9306042. ISSN: 1019-6439. Pub. country: Greece. Language:
English.

- AB Receptors for luteinizing hormone-releasing hormone (LHRH), expressed by ovarian cancers, can be used for targeting chemotherapeutic compounds more selectively to these tumors. We investigated the effects of cytotoxic LHRH analog AN-152, consisting of doxorubicin (DOX)-14-O-hemiglutarate linked to the epsilon-amino group of [D-Lys6]LHRH, on the growth of LHRH receptor-positive ES-2 human ovarian cancer line xenografted into nude mice. A single injection of AN-152, at a dose of 345 nmol/20 g body weight, caused a 34.5% reduction ($P<0.05$) in tumor growth after 28 days, while its cytotoxic moiety DOX was inactive at the same dose. Since the overexpression of certain growth factors and/or their receptors, such as vascular endothelial growth factor (VEGF), epidermal growth factor receptor (EGFR) and HER-2/neu, as well as various oncogenes like c-fos and c-jun, is associated with unfavorable prognosis and contributes to progressive growth of ovarian carcinomas, their mRNA levels were analyzed by RT-PCR. Treatment with AN-152 significantly ($P<0.05$) reduced the expression of EGFR, **VEGF**, c-fos and c-jun, to 49%, 48%, 55% and 58% respectively, compared to controls. HER-2/neu mRNA expression was also decreased to non-detectable levels. Conversely, DOX decreased non-significantly the expression levels for EGFR by 32%, VEGF 35%, both c-fos and c-jun approximately 20% and HER-2/neu by only 15%. In conclusion, cytotoxic LHRH analog AN-152 could be considered for chemotherapy of ovarian cancers expressing LHRH receptors.

L12 ANSWER 18 OF 20 MEDLINE on STN DUPLICATE 1
1998325020. PubMed ID: 9660776. p42/p44 MAP kinase module plays a key role in the transcriptional regulation of the vascular endothelial growth factor gene in fibroblasts. Milanini J; Vinals F; Pouyssegur J; Pages G. (Centre de Biochimie, CNRS-UMR 6543, Universite de Nice, Parc Valrose, 06108 Nice, France.) The Journal of biological chemistry, (1998 Jul 17) Vol. 273, No. 29, pp. 18165-72. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

- AB Vascular Endothelial Growth Factor (VEGF) is a potent mitogen for vascular endothelial cells that has been implicated in tumor neovascularization. We show that, in hamster fibroblasts (CCL39 cells), VEGF mRNAs are expressed at low levels in serum-deprived or exponentially growing cells, whereas it is rapidly induced after stimulation of quiescent cells with serum. CCL39 **derivatives**, transformed with Polyoma virus or with active members of the p42/p44 mitogen-activated protein (MAP) kinase pathway, Gly/Val point mutant of Ras at position 12 (Ras-Vall2), MKK1 in which Ser218 and Ser222 were mutated to Asp (MKK1-SS/DD), express very high levels of VEGF mRNA. To analyze the contribution of the p42/p44MAP kinase in this induction, we used the CCL39-derived cell line (Raf-1:ER) expressing an estradiol-activable Raf-1. We show a time and an estradiol dose-dependent up-regulation of VEGF mRNA clearly detectable after 2 h of stimulation. The induction of VEGF mRNA in response to conditioned activation of Raf-1 is reverted by an inhibitor of MKK1, PD 098059, highlighting a specific role for the p42/p44 MAP kinase pathway in VEGF expression. Interestingly, hypoxia has an additive effect on VEGF induction in CCL39 cells stimulated by serum or in Raf-1:ER cells stimulated by estradiol. In contrast to VEGF, the isoforms VEGF-B and **VEGF-C** are poorly regulated by growth and oncogenic factors. We have identified a GC-rich region of the VEGF promoter between -88 and -66 base pairs which contains all the elements responsible of its up-regulation by constitutive active Ras or MKK1-SS/DD. By mutation of the putative binding sites and electrophoretic mobility supershift experiments, we showed that the GC-rich region constitutively binds Sp1 and AP-2 transcription factors. Furthermore, following activation of the

p42/p44 MAP kinase module, the binding of Sp1 and AP-2 is increased in the complexes formed in this region of the promoter. Altogether, these data suggest that hypoxia and p42/p44 MAP kinase independently play a key role in the regulation of the VEGF expression.

- L12 ANSWER 19 OF 20 MEDLINE on STN DUPLICATE 2
1998118549. PubMed ID: 9435229. Vascular endothelial growth factor D (VEGF-D) is a ligand for the tyrosine kinases VEGF receptor 2 (Flk1) and VEGF receptor 3 (Flt4). Achen M G; Jeltsch M; Kukk E; Makinen T; Vitali A; Wilks A F; Alitalo K; Stacker S A. (Ludwig Institute for Cancer Research, Royal Melbourne Hospital, Victoria, Australia.. Marc.achen@ludwig.edu.au) . Proceedings of the National Academy of Sciences of the United States of America, (1998 Jan 20) Vol. 95, No. 2, pp. 548-53. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.
- AB We have identified a member of the VEGF family by computer-based homology searching and have designated it VEGF-D. VEGF-D is most closely related to **VEGF-C** by virtue of the presence of N- and C-terminal extensions that are not found in other VEGF family members. In adult human tissues, VEGF-D mRNA is most abundant in heart, lung, skeletal muscle, colon, and small intestine. Analyses of VEGF-D receptor specificity revealed that VEGF-D is a ligand for both VEGF receptors (VEGFRs) VEGFR-2 (Flk1) and VEGFR-3 (Flt4) and can activate these receptors. However. VEGF-D does not bind to VEGFR-1. Expression of a truncated **derivative** of VEGF-D demonstrated that the receptor-binding capacities reside in the portion of the molecule that is most closely related in primary structure to other VEGF family members and that corresponds to the mature form of **VEGF-C**. In addition, VEGF-D is a mitogen for endothelial cells. The structural and functional similarities between VEGF-D and **VEGF-C** define a subfamily of the VEGFs.

- L12 ANSWER 20 OF 20 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN
1996:866804 The Genuine Article (R) Number: VV760. Crystallization of the receptor binding domain of vascular endothelial growth factor. Christinger H W (Reprint); Muller Y A; Berleau L T; Keyt B A; Cunningham B C; Ferrara N; deVos A M. GENENTECH INC, DEPT PROT ENGN, S SAN FRANCISCO, CA 94080; GENENTECH INC, DEPT CARDIOVASC RES, S SAN FRANCISCO, CA 94080. PROTEINS-STRUCTURE FUNCTION AND GENETICS (NOV 1996) Vol. 26, No. 3, pp. 353-357. ISSN: 0887-3585. Publisher: WILEY-LISS, DIV JOHN WILEY & SONS INC 605 THIRD AVE, NEW YORK, NY 10158-0012. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

- AB Vascular endothelial growth factor (VEGF) is a potent angiogenic factor with a unique specificity for vascular endothelial cells. In addition to its role in vasculogenesis and embryonic angiogenesis, VEGF is implicated in pathologic neovascularization associated with tumors and diabetic retinopathy. Four different constructs of a short variant of VEGF sufficient for receptor binding were overexpressed in Escherichia coli, refolded, purified, and crystallized in five different space groups. In order to facilitate the production of heavy atom **derivatives**, single cysteine mutants were designed based on the crystal structure of platelet-derived growth factor. A construct consisting of residues 8 to 109 was crystallized in space group P2(1), with cell parameters a = 55.6 Angstrom, b = 60.4 Angstrom, c = 77.7 Angstrom, beta = 90.0 degrees, and four monomers in the asymmetric unit. Native and **derivative** data were collected for two of the cysteine mutants as well as for wild-type **VEGF**. (C) 1996 Wiley-Liss, Inc.

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FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 17:33:48 ON 03 MAY 2006

L1 0 S VEGFR3 AGONIST
L2 0 S VEGF RECEPTOR 3 AGONIST
L3 0 S VEGF RECEPTOR 3 ANTAGONIST
L4 341 S VEGFR3
L5 55 S L4 AND LIGAND
L6 28 DUP REMOVE L5 (27 DUPLICATES REMOVED)
L7 2842 S VEGF-C
L8 95 S L7 AND ANTAGONIST
L9 4 S L8 AND DERIVATIVE
L10 4 DUP REMOVE L9 (0 DUPLICATES REMOVED)
L11 28 S L7 AND DERIVATIVE
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L13 26 L7 AND DIMER

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L14 ANSWER 1 OF 10 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN

2005135325 EMBASE Vascular endothelial growth factor (VEGF)-A(165)-induced prostacyclin synthesis requires the activation of VEGF receptor-1 and -2 heterodimer. Neagoe P.-E.; Lemieux C.; Sirois M.G.. M.G. Sirois, Research Center, Montreal Heart Institute, 5000 Belanger St., Montreal, Que. H1T 1C8, Canada. martin.sirois@icm-mhi.org. Journal of Biological Chemistry Vol. 280, No. 11, pp. 9904-9912 18 Mar 2005.

Refs: 54.

ISSN: 0021-9258. CODEN: JBCHA3

Pub. Country: United States. Language: English. Summary Language: English. Entered STN: 20050414. Last Updated on STN: 20050414

AB We previously reported that vascular endothelial growth factor (VEGF)-A(165) inflammatory effect is mediated by acute platelet-activating factor synthesis from endothelial cells upon the activation of VEGF receptor-2 (VEGFR-2) and its coreceptor, neuropilin-1 (NRP-1). In addition, VEGF-A(165) promotes the release of other endothelial mediators including nitric oxide and prostacyclin (PGI(2)). However, it is unknown whether VEGF-A(165) is mediating PGI(2) synthesis through VEGF receptor-1 (VEGFR-1) and/or VEGF receptor-2 (VEGFR-2) activation and whether the coreceptor NRP-1 potentiates VEGF-A(165) activity. In this study, PGI(2) synthesis in bovine aortic endothelial cells (BAEC) was assessed by quantifying its stable metabolite (6-keto prostaglandin F (1 α), 6-keto PGF(1 α)) by enzyme-linked immunosorbent assay. Treatment of BAEC with VEGF analogs, VEGF-A(165) (VEGFR-1, VEGFR-2 and NRP-1 agonist) and VEGF-A(121) (VEGFR-1 and VEGFR-2 agonist) (up to 10(-9) M), increased PGI(2) synthesis by 70- and 40-fold within 15 min. Treatment with VEGFR-1 (placental growth factor and VEGF-B) or VEGFR-2 (**VEGF-C**) agonist did not increase PGI(2) synthesis. The combination of VEGFR-1 and VEGFR-2 agonists did not increase PGI(2) release. Pretreatment with a VEGFR-2 inhibitor abrogated PGI(2) release mediated by VEGF-A(165) and VEGF-A (121), and pre-treatment of BAEC with antisense oligomers targeting VEGFR-1 or VEGFR-2 mRNA reduced PGI(2) synthesis mediated by VEGF-A(165) and VEGF-A(121) up to 79%. In summary, our data demonstrate that the

activation of VEGFR-1 and VEGFR-2 heterodimer (VEGFR-1/R-2) is essential for PGI(2) synthesis mediated by VEGF-A(165) and VEGF-A(121), which cannot be reproduced by the parallel activation of VEGFR-1 and VEGFR-2 homodimers with corresponding agonists. In addition, the binding of VEGF-A(165) to NRP-1 potentiates its capacity to promote PGI(2) synthesis. .COPYRGT. 2005 by The American Society for Biochemistry and Molecular Biology, Inc.

=> s 17 and cyclic

L15 28 L7 AND CYCLIC

=> dup remove l15

PROCESSING COMPLETED FOR L15

L16 11 DUP REMOVE L15 (17 DUPLICATES REMOVED)

=> d l16 1-11 cbib abs

L16 ANSWER 1 OF 11 MEDLINE on STN. DUPLICATE 1

2005669032. PubMed ID: 16357152. gamma-Aminobutyric acid inhibits cholangiocarcinoma growth by **cyclic** AMP-dependent regulation of the protein kinase A/extracellular signal-regulated kinase 1/2 pathway. Fava Giammarco; Marucci Luca; Glaser Shannon; Francis Heather; De Morrow Sharon; Benedetti Antonio; Alvaro Domenico; Venter Julie; Meininger Cynthia; Patel Tushar; Taffetani Silvia; Marzioni Marco; Summers Ryun; Reichenbach Ramona; Alpini Gianfranco. (Central Texas Veterans Health Care System, Research Service, College of Medicine, Temple, 76504, USA.. galpini@tamu.edu) . Cancer research, (2005 Dec 15) Vol. 65, No. 24, pp. 11437-46. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB We studied the effect of the inhibitory neurotransmitter, gamma-aminobutyric acid (GABA), in the regulation of cholangiocarcinoma growth. We determined the in vitro effect of GABA on the proliferation of the cholangiocarcinoma cell lines (Mz-ChA-1, HuH-28, and TFK-1) and evaluated the intracellular pathways involved. The effect of GABA on migration of Mz-ChA-1 cells was also evaluated. In vivo, Mz-ChA-1 cells were s.c. injected in athymic mice, and the effects of GABA on tumor size, tumor cell proliferation, apoptosis, collagen quantity, and the expression of vascular endothelial growth factor-A (VEGF-A) and **VEGF-C** (cancer growth regulators) were measured after 82 days. GABA decreased in vitro cholangiocarcinoma growth in a time-dependent and dose-dependent manner, by both **cyclic** AMP/protein kinase A- and D-myo-inositol-1,4,5-thriphosphate/Ca(2+)-dependent pathways, leading to down-regulation of extracellular signal-regulated kinase 1/2 phosphorylation. Blocking of GABA(A), GABA(B), and GABA(C) receptors prevented GABA inhibition of cholangiocarcinoma proliferation. GABA inhibited Mz-ChA-1 cell migration and, in vivo, significantly decreased tumor volume, tumor cell proliferation, and VEGF-A/C expression whereas increasing apoptosis compared with controls. An increase in collagen was evident in GABA-treated tumors. GABA decreases biliary cancer proliferation and reduces the metastatic potential of cholangiocarcinoma. GABA may represent a therapeutic agent for patients affected by malignancies of the biliary tract.

L16 ANSWER 2 OF 11 MEDLINE on STN. DUPLICATE 2

2005458522. PubMed ID: 16102752. Modulating furin activity with designed mini-PDX peptides: synthesis and in vitro kinetic evaluation. Basak Ajoy; Lotfipour Farzaneh. (Diseases of Aging Program, Regional Protein Chemistry Center, Ottawa Health Research Institute, University of Ottawa, Loeb Building, 725 Parkdale Ave, Ottawa, ON, K1Y 4E9, Canada.. abasak@ohri.ca) . FEBS letters, (2005 Aug 29) Vol. 579, No. 21, pp. 4813-21. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB A peptide was designed from reactive site loop structure of alpha1

Antitrypsin Portland known as alpha1 PDX as a novel mini-PDX inhibitor of furin. The sequence was derived from (367-394) that contains the crucial furin cleavage motif R1PR382. A P3 mutant replacing Ile380 by Leu was prepared as a first model peptide. A Cys residue was inserted at each terminal of the peptide for purpose of cyclisation which was accomplished by air or iodine-induced oxidation. This mini-PDX peptide both **cyclic** and acyclic form inhibited in vitro furin activity (IC50 in nM) when measured against either substrates Boc-RVRRdown double arrow MCA or QVEGF-C [Abz-QVHSIIRRdown double arrow SLP-Y(NO2)-A-CONH2, Abz=2-amino benzoic acid and Y(NO2)=3-nitro tyrosine], latter being derived from vascular endothelial growth factor-C (**VEGF-C**) processing site. The geometrically constrained structure mimicking PDX reactive loop is crucial for enzyme inhibition. Our study further revealed that both mini-PDX peptides inactivate furin in a slow tight binding manner, with disulfide-bridged **cyclic** form being slightly more potent. Unlike PDX, these peptides inhibit furin via a different mechanistic pathway. The study provides an alternate strategy for development of efficient peptide-based inhibitors of Proprotein Convertases including furin.

- L16 ANSWER 3 OF 11 MEDLINE on STN DUPLICATE 3
 2005040392. PubMed ID: 15668894. Vascular endothelial growth factor A and C gene expression in endometriosis. Takehara Mikio; Ueda Masatsugu; Yamashita Yoshiki; Terai Yoshito; Hung Yao-Ching; Ueki Minoru. (Department of Obstetrics and Gynecology, Osaka Medical College, Japan.) Human pathology, (2004 Nov) Vol. 35, No. 11, pp. 1369-75. Journal code: 9421547. ISSN: 0046-8177. Pub. country: United States. Language: English.
- AB Angiogenesis is essential for the pathogenesis of endometriosis. Gene expression levels of vascular endothelial growth factor (VEGF) A and C in 10 eutopic endometrial, 23 normal peritoneal, and 62 endometriotic tissues surgically obtained from 47 women with endometriosis (group 2) were compared with those in 12 control eutopic endometrial and 9 normal peritoneal tissues from 15 women without endometriosis (group 1). VEGF-A mRNA expression levels in eutopic endometrium of group 2 were higher than those of group 1 throughout the menstrual cycle (P <0.01) and increased in the secretory phase. VEGF-A gene expression in peritoneal endometriotic lesion was statistically higher than that in normal peritoneum (P <0.01) and similar to that in eutopic endometrium of group 2. In contrast, gene expression levels of **VEGF-C** were relatively lower than those of VEGF-A in each lesion, and no **cyclic** variation was found. VEGF-A and C mRNA expression levels were significantly higher in ovarian endometriomas >6 cm in size than in those <6 cm in size. Immunohistochemical expression of VEGF-A and C was detected in the cytoplasm of glandular epithelial and stromal cells of ovarian endometrioma. These results suggest that endometriosis may arise from eutopic endometrium with higher levels of angiogenic activity possibly induced by VEGF-A in women with endometriosis. Moreover, **VEGF-C** as well as VEGF-A may be involved in the pathogenesis of ovarian endometrioma.

- L16 ANSWER 4 OF 11 MEDLINE on STN DUPLICATE 4
 2004010164. PubMed ID: 14707101. Prostaglandin E2 induces degranulation-independent production of vascular endothelial growth factor by human mast cells. Abdel-Majid Raja M; Marshall Jean S. (Department of Pathology, Dalhousie University, College Street, Halifax, Nova Scotia B3H 4H7, Canada.) Journal of immunology (Baltimore, Md. : 1950), (2004 Jan 15) Vol. 172, No. 2, pp. 1227-36. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.
- AB Mast cells accumulate in large numbers at angiogenic sites, where they have been shown to express a number of proangiogenic factors, including vascular endothelial growth factor (VEGF-A). PGE(2) is known to strongly promote angiogenesis and is found in increased levels at sites of chronic inflammation and around solid tumors. The expression pattern of VEGF and

the regulation of VEGF-A by PGE(2) were examined in cord blood-derived human mast cells (CBMC). CBMC expressed mRNA for five isoforms of VEGF-A and other members of the VEGF family (VEGF-B, **VEGF-C**, and VEGF-D) with strong expression of the most potent secretory isoforms. PGE(2) was a very strong inducer of VEGF-A(121/165) production by CBMC and also elevated VEGF-A mRNA expression. The amount of VEGF-A(121/165) protein production induced by PGE(2) was 4-fold greater than that induced by IgE-mediated activation of CBMC. Moreover, the response to PGE(2) as well as to other cAMP-elevating agents such as forskolin and salbutamol was observed under conditions that were not associated with mast cell degranulation. CBMC expressed substantial levels of the EP(2) receptor, but not the EP(4) receptor, when examined by flow cytometry. In contrast to other reported PGE(2)-mediated effects on mast cells, VEGF-A(121/165) production occurred via activation of the EP(2) receptor. These data suggest a role for human mast cells as a potent source of VEGF(121/165) in the absence of degranulation, and may provide new opportunities to regulate angiogenesis at mast cell-rich sites.

L16 ANSWER 5 OF 11 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

2003:452398 The Genuine Article (R) Number: 658QZ. **VEGF-C** mediates the proliferative response of endothelial cells to **cyclic** pressure. Shin H W (Reprint); Smith M; Toy K; Williams M; Bizios R; Gerritsen M. Rensselaer Polytech Inst, Troy, NY 12180 USA; Univ Virginia, Charlottesville, VA USA; Genentech Inc, San Francisco, CA 94080 USA. FASEB JOURNAL (14 MAR 2003) Vol. 17, No. 4, Part 1, Supp. [S], pp. A531-A532. ISSN: 0892-6638. Publisher: FEDERATION AMER SOC EXP BIOL, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3998 USA. Language: English.

L16 ANSWER 6 OF 11 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN DUPLICATE 5

2003376877 EMBASE **VEGF-C** mediates **cyclic** pressure-induced endothelial cell proliferation. Shin H.Y.; Smith M.L.; Toy K.J.; Williams P.M.; Bizios R.; Gerritsen M.E.. R. Bizios, Dept. of Biomedical Engineering, Rensselaer Polytechnic Institute, Jonsson Engineering Center, 110 8th St., Troy, NY 12180-3590, United States. bizios@rpi.edu. Physiological Genomics Vol. 11, pp. 245-251 2003. Refs: 30. ISSN: 1531-2267. CODEN: PHGEFP. Pub. Country: United States. Language: English. Summary Language: English. Entered STN: 20031002. Last Updated on STN: 20031002

AB Mechanical forces modulate endothelial cell functions through several mechanisms including regulation of gene transcription. In the present study, gene transcription by human umbilical vein endothelial cells (HUVEC) either maintained under control pressure (that is, standard cell culture conditions equivalent to 0.15 mmHg sustained hydrostatic pressure) or exposed to 60/20 mmHg sinusoidal pressures at 1 Hz were compared using Affymetrix GeneChip microarrays to identify cellular/molecular mechanisms associated with endothelial cell responses to **cyclic** pressure. **Cyclic** pressure selectively affected transcription of 14 genes that included a set of mechanosensitive proteins involved in hemostasis (tissue plasminogen activator), cell adhesion (integrin- α (2)), and cell signaling (Rho B, cytosolic phospholipase A(2)), as well as a unique subset of **cyclic** pressure-sensitive genes such as vascular endothelial growth factor (**VEGF**)-C and transforming growth factor (TGF)- β (2). The present study also provided first evidence that **VEGF-C**, the most highly induced gene under 60/20 mmHg, mediated HUVEC proliferation in response to this **cyclic** pressure. **Cyclic** pressure is, therefore, a mechanical force that modulates endothelial cell functions (such as proliferation) by activating a specific transcriptional program.

L16 ANSWER 7 OF 11 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

2003:410839 Document No.: PREV200300410839. **VEGF-C**

mediates the proliferative response of endothelial cells to **cyclic** pressure. Shin, Hainsworth [Reprint Author]; Smith, Michael; Toy, Karen; Williams, Mickey; Bizios, Rena; Gerritsen, Mary. Biomedical Engineering, Rensselaer Polytechnic Institute, 110 8th St., Troy, NY, 12180, USA. shinh@rpi.edu; mls3d@cms.mail.virginia.edu; toy@gene.com; mickey@gene.com; bizios@rpi.edu; mary.gerritsen@mpi.com. FASEB Journal, (March 2003) Vol. 17, No. 4-5, pp. Abstract No. 344.25. <http://www.fasebj.org/>. e-file. Meeting Info.: FASEB Meeting on Experimental Biology: Translating the Genome. San Diego, CA, USA. April 11-15, 2003. FASEB. ISSN: 0892-6638 (ISSN print). Language: English.

AB Fluid pressure is an independent mechanical stimulus to which endothelial cells are exposed in vivo; the effects of **cyclic** pressure on endothelial cell function, however, remain poorly characterized. We previously reported that exposure of human umbilical vein endothelial cells (HUVEC) to 60/20 mm Hg sinusoidal pressure stimulated cell proliferation. In order to identify the mediator(s) of this response, the present study used Affymetrix oligonucleotide microarrays to investigate the transcriptional responses of HUVEC to 60/20 mm Hg **cyclic** pressure at 1 Hz for 24 hours. Fourteen pressure-sensitive genes were identified, including those involved in angiogenesis (vascular endothelial growth factor-C, or **VEGF-C**, and interleukin-8), hemostasis (tissue plasminogen activator), cell adhesion (integrin (2), cell differentiation (transforming growth factor-(2) and cell signaling (Rho B and cytosolic phospholipase A2). Of these, **VEGF-C**, a potent endothelial mitogen, was the most highly-induced gene. Moreover, using soluble **VEGF-C** receptor chimera (flt-4/IgG) and blocking **VEGF-C** antibodies, we showed that **VEGF-C** mediates the **cyclic** pressure-induced proliferation. The gene transcription profile elucidated by the present study, therefore, provides critical new information regarding the potential pathways linking **cyclic** pressure and endothelial cell function(s) pertinent to vascular physiology.

L16 ANSWER 8 OF 11 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

2002:575139 The Genuine Article (R) Number: 567QG. A tumor-homing peptide with a targeting specificity related to lymphatic vessels. Laakkonen P; Porkka K; Hoffman J A; Ruoslahti E (Reprint). Burnham Inst, Canc Res Ctr, La Jolla, CA 92037 USA (Reprint); Univ Calif San Diego, Sch Med, Burnham Inst, Program Mol Pathol, La Jolla, CA 92093 USA; Univ Calif San Diego, Sch Med, Dept Pathol, La Jolla, CA 92093 USA. NATURE MEDICINE (JUL 2002) Vol. 8, No. 7, pp. 751-755. ISSN: 1078-8956. Publisher: NATURE AMERICA INC , 345 PARK AVE SOUTH, NEW YORK, NY 10010-1707 USA. Language: English. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Blood vessels of tumors carry specific markers that are usually angiogenesis-related(1,2). We previously used phage-displayed peptide libraries in vivo to identify peptides that home to tumors through the circulation and that specifically bind to the endothelia of tumor blood vessels(3,4). Here we devised a phage screening procedure that would favor tumor-homing to targets that are accessible to circulating phage, but are not blood vessels. Screening on MDA-MB-435 breast carcinoma xenografts yielded multiple copies of a phage that displays a **cyclic** 9-amino-acid peptide, LyP-1. Homing and binding to tumor-derived cell suspensions indicated that LyP-1 also recognizes an osteosarcoma xenograft, and spontaneous prostate and breast cancers in transgenic mice, but not two other tumor xenografts. Fluorescein-labeled LyP-1 peptide was detected in tumor structures that were positive for three lymphatic endothelial markers and negative for three blood vessel markers. LyP-1 accumulated in the nuclei of the putative lymphatic cells, and in the nuclei of tumor cells. These results suggest that tumor lymphatics carry specific markers and that it may be possible to specifically target therapies into tumor lymphatics.

2002703381. PubMed ID: 12388793. **VEGF-C** mediates

cyclic pressure-induced endothelial cell proliferation. Shin Hainsworth Y; Smith Michael L; Toy Karen J; Williams P Mickey; Bizios Rena; Gerritsen Mary E. (Department of Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, New York 12180-3590, USA.) Physiological genomics, (2002 Dec 3) Vol. 11, No. 3, pp. 245-51. Electronic Publication: 2002-12-03. Journal code: 9815683. E-ISSN: 1531-2267. Pub. country: United States. Language: English.

AB Mechanical forces modulate endothelial cell functions through several mechanisms including regulation of gene transcription. In the present study, gene transcription by human umbilical vein endothelial cells (HUVEC) either maintained under control pressure (that is, standard cell culture conditions equivalent to 0.15 mmHg sustained hydrostatic pressure) or exposed to 60/20 mmHg sinusoidal pressures at 1 Hz were compared using Affymetrix GeneChip microarrays to identify cellular/molecular mechanisms associated with endothelial cell responses to **cyclic** pressure. **Cyclic** pressure selectively affected transcription of 14 genes that included a set of mechanosensitive proteins involved in hemostasis (tissue plasminogen activator), cell adhesion (integrin-alpha2), and cell signaling (Rho B, cytosolic phospholipase A2), as well as a unique subset of **cyclic** pressure-sensitive genes such as vascular endothelial growth factor (**VEGF**)-C and transforming growth factor (TGF)-beta2. The present study also provided first evidence that **VEGF-C**, the most highly induced gene under 60/20 mmHg, mediated HUVEC proliferation in response to this **cyclic** pressure. **Cyclic** pressure is, therefore, a mechanical force that modulates endothelial cell functions (such as proliferation) by activating a specific transcriptional program.

L16 ANSWER 10 OF 11 CAPLUS COPYRIGHT 2006 ACS on STN

2001:545508 Document No. 135:132464 **Cyclic** peptide inhibitors of

VEGF, **VEGF-C**, and VEGF-D, preparation methods, pharmaceutical compositions, and therapeutic use. Achen, Marc G.; Hughes, Richard A.; Stacker, Steven; Cendron, Angela (Ludwig Institute for Cancer Research, USA). PCT Int. Appl. WO 2001052875 A1 20010726, 102 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US1533 20010118. PRIORITY: US 2000-PV176293 20000118; US 2000-PV204590 20000516.

AB The invention provides monomeric monocyclic peptide inhibitors and dimeric bicyclic peptide inhibitors based on exposed loop fragments of a growth factor protein, e.g. loop 1, loop 2 or loop 3 of VEGF-D, as well as methods of making them, pharmaceutical compns. containing them, and therapeutic methods of use.

L16 ANSWER 11 OF 11 MEDLINE on STN

DUPLICATE 7

1998006425. PubMed ID: 9348202. Differential hormonal regulation of vascular endothelial growth factors VEGF, VEGF-B, and **VEGF-C**

messenger ribonucleic acid levels in cultured human granulosa-luteal cells. Laitinen M; Ristimaki A; Honkasalo M; Narko K; Paavonen K; Ritvos O. (Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki, Finland.. mplaitin@cc.helsinki.fi) . Endocrinology, (1997 Nov) Vol. 138, No. 11, pp. 4748-56. Journal code: 0375040. ISSN: 0013-7227. Pub. country: United States. Language: English.

AB The development of ovarian follicles and subsequent corpus luteum formation is accompanied by very active angiogenesis. Ovarian granulosa

cells produce vascular endothelial growth factor (VEGF), which is a potent endothelial cell mitogen and an angiogenic agent. The complementary DNAs of two other factors structurally related to VEGF, namely VEGF-B and **VEGF-C**, were recently cloned, but little is known of their regulation in the ovary. We first studied the expression of the messenger RNAs (mRNAs) of the three VEGF isoforms in freshly isolated human granulosa-luteal (GL) cells obtained at oocyte retrieval for in vitro fertilization. The hormonal regulation of these mRNAs was subsequently studied in primary cultures of human GL cells. Analysis of cultured GL cell RNA by reverse transcription-PCR revealed that these cells express the alternatively spliced transcripts representing 121-, 145-, and 165-amino acid VEGF isoforms. Northern blot hybridization analyses indicated that transcripts of 4.5 and 3.7 kilobases for VEGF, and 1.4 and 2.4 kilobases for VEGF-B and **VEGF-C**, respectively, are expressed in human GL cells. The basal VEGF mRNA levels declined steadily, whereas VEGF-B mRNA levels were rather invariant over a 10-day culture period of GL cells. In contrast, **VEGF-C** mRNA levels increased toward the end of culture. For studying the hormonal regulation of VEGF isotype mRNAs, GL cells were treated with hCG, recombinant human FSH, PGE2, as well as 8-bromo-cAMP and 12-O-tetradecanoylphorbol 13-acetate, which activate protein kinase A- and protein kinase C-dependent signaling pathways, respectively. All test agents stimulated the expression of VEGF mRNA levels in a concentration-dependent manner. Time-course studies indicated that all treatments induced VEGF mRNA levels as early as incubation for 2 h, and the effect was sustained up to 48 h. VEGF-B mRNA levels were not regulated by any of the test agents. However, we found that hCG and 8-bromo-cAMP decreased **VEGF-C** mRNA levels with a maximal response observed at 24 and 48 h after cellular treatment. We conclude that the mRNAs of VEGF, VEGF-B, and **VEGF-C** are expressed in human GL cells and that their mRNA steady state levels are regulated in cultured human GL cells in an isotype-specific manner. The differential regulation of VEGF, VEGF-B, and **VEGF-C** in human GL cells suggests that distinct VEGF isoforms may play different roles during the vascularization of the human ovarian follicle and corpus luteum.

=> s cyclic peptide

L17 13602 CYCLIC PEPTIDE

=> s l17 and binds VEGF receptor 3

L18 0 L17 AND BINDS VEGF RECEPTOR 3

=> s l17 and VEGF receptor

L19 12 L17 AND VEGF RECEPTOR

=> dup remove l19

PROCESSING COMPLETED FOR L19

L20 5 DUP REMOVE L19 (7 DUPLICATES REMOVED)

=> d l20 1-5 cbib abs

L20 ANSWER 1 OF 5 MEDLINE on STN

DUPLICATE 1

2005565108. PubMed ID: 16242650. Structural basis for the interaction of a vascular endothelial growth factor mimic peptide motif and its corresponding receptors. Giordano Ricardo J; Anobom Cristiane D; Cardo-Vila Marina; Kalil Jorge; Valente Ana P; Pasqualini Renata; Almeida Fabio C L; Arap Wadih. (The University of Texas, MD Anderson Cancer Center, Houston, 77030, USA.) Chemistry & biology, (2005 Oct) Vol. 12, No. 10, pp. 1075-83. Journal code: 9500160. ISSN: 1074-5521. Pub. country: England: United Kingdom. Language: English.

AB Vascular endothelial growth factor (VEGF) is central to the survival and

development of the vascular and nervous systems. We screened phage display libraries and built a peptide-based ligand-receptor map of binding sites within the VEGF family. We then validated a **cyclic peptide**, CPQPRPLC, as a VEGF-mimic that binds specifically to neuropilin-1 and **VEGF receptor-1**. Here, we use NMR spectroscopy to understand the structural basis of the interaction between our mimic peptide and the **VEGF receptors**. We show that: (1) CPQPRPLC has multiple interactive conformations; (2) receptor binding is mediated by the motif Arg-Pro-Leu; and (3) the Pro residue within Arg-Pro-Leu participates in binding to neuropilin-1 but not to **VEGF receptor-1**, perhaps representing an evolutionary gain-of-function. Therefore, Arg-Pro-Leu is a differential ligand motif to **VEGF receptors** and a candidate peptidomimetic lead for VEGF pathway modulation.

- L20 ANSWER 2 OF 5 MEDLINE on STN DUPLICATE 2
 2003437784. PubMed ID: 12837752. Structure and inhibitory effects on angiogenesis and tumor development of a new vascular endothelial growth inhibitor. Zilberberg Lior; Shinkaruk Svetlana; Lequin Olivier; Rousseau Benoit; Hagedorn Martin; Costa Francesco; Caronzolo Dario; Balke Maurice; Canron Xavier; Convert Odile; Lain Georges; Gionnet Karine; Goncalves Mario; Bayle Mireille; Bello Lorenzo; Chassaing Gerard; Deleris Gerard; Bikfalvi Andreas. (Molecular Angiogenesis Laboratory, INSERM E 0113, Universite de Bordeaux 1, 33405 Talence, France.) The Journal of biological chemistry, (2003 Sep 12) Vol. 278, No. 37, pp. 35564-73. Electronic Publication: 2003-07-01. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.
- AB Blocking angiogenesis is an attractive strategy to inhibit tumor growth, invasion, and metastasis. We describe here the structure and the biological action of a new **cyclic peptide** derived from vascular endothelial growth factor (VEGF). This 17-amino acid molecule designated cyclopeptidic vascular endothelial growth inhibitor (cyclo-VEGI, CBO-P11) encompasses residues 79-93 of VEGF which are involved in the interaction with **VEGF receptor-2**. In aqueous solution, cyclo-VEGI presents a propensity to adopt a helix conformation that was largely unexpected because only beta-sheet structures or random coil conformations have been observed for macrocyclic peptides. Cyclo-VEGI inhibits binding of iodinated VEGF165 to endothelial cells, endothelial cells proliferation, migration, and signaling induced by VEGF165. This peptide also exhibits anti-angiogenic activity in vivo on the differentiated chicken chorioallantoic membrane. Furthermore, cyclo-VEGI significantly blocks the growth of established intracranial glioma in nude and syngeneic mice and improves survival without side effects. Taken together, these results suggest that cyclo-VEGI is an attractive candidate for the development of novel angiogenesis inhibitor molecules useful for the treatment of cancer and other angiogenesis-related diseases.

- L20 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2006 ACS on STN
 2003:157842 Document No. 139:385998 Peptide-targeted PEG-liposomes in anti-angiogenic therapy. Janssen, A. P. C. A.; Schiffelers, R. M.; ten Hagen, T. L. M.; Koning, G. A.; Schraa, A. J.; Kok, R. J.; Storm, G.; Molema, G. (Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht, Neth.). International Journal of Pharmaceutics, 254(1), 55-58 (English) 2003. CODEN: IJPHDE. ISSN: 0378-5173. Publisher: Elsevier Science B.V..
- AB Peptides with the RGD amino acid sequence show affinity for the alpha(v)beta(3) integrin, an integrin which is over-expressed on angiogenic endothelium and involved in cell adhesion. A peptide with the sequence ATWLPPR was demonstrated to show affinity for the vascular endothelial growth factor (**VEGF**) **receptor**, a receptor involved in the proliferation of endothelial cells. By coupling these peptides to liposomes, these liposomes can serve as a site-specific drug

delivery system to tumor endothelial cells to inhibit angiogenesis. In the present study the authors demonstrate that the coupling of cyclic RGD-peptides or ATWLPPR-peptides to the surface of PEG-liposomes results in binding of these liposomes to endothelial cells in vitro. Subsequent studies with RGD-peptide targeted liposomes in vivo also demonstrate specific binding to the tumor endothelium.

L20 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2006 ACS on STN

2001:816859 Document No. 135:366772 Peptide compounds having affinity for the vascular endothelial growth factor receptor-2 (VEGFR-2) and associated uses. Schatz, Peter Joseph; Chen, Min-Jia; Piplani, Sunila; Mozsgai, Cecilia A.; Balu, Palani (Glaxo Group Limited, UK). PCT Int. Appl. WO 2001083693 A2 20011108, 101 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US13598 20010427. PRIORITY: US 2000-561470 20000428.

AB Compds. are provided that bind to VEGFR-2. The compds. have a peptide chain approx. 8-40 amino acids in length that binds to VEGFR-2, or are dimers of such peptide chains. The compds. are useful as probes for affinity screening and as angiogenesis imaging agents. In addition, those compds. that are antagonists of VEGFR-2 are useful in the treatment of diseases including cancer, retinopathy, rheumatoid arthritis and others. Pharmaceutical compns. and methods of use are provided as well.

L20 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2006 ACS on STN

2001:545508 Document No. 135:132464 **Cyclic peptide** inhibitors of VEGF, VEGF-C, and VEGF-D, preparation methods, pharmaceutical compositions, and therapeutic use. Achen, Marc G.; Hughes, Richard A.; Stacker, Steven; Cendron, Angela (Ludwig Institute for Cancer Research, USA). PCT Int. Appl. WO 2001052875 A1 20010726, 102 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US1533 20010118. PRIORITY: US 2000-PV176293 20000118; US 2000-PV204590 20000516.

AB The invention provides monomeric monocyclic peptide inhibitors and dimeric bicyclic peptide inhibitors based on exposed loop fragments of a growth factor protein, e.g. loop 1, loop 2 or loop 3 of VEGF-D, as well as methods of making them, pharmaceutical compns. containing them, and therapeutic methods of use.

=> s CGYWLTIWGC

L21 1 CGYWLTIWGC

=> d l21 cbib abs

L21 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2006 ACS on STN

2002:555522 Document No. 137:119669 VEGFR-3 inhibitor materials and methods. Alitalo, Kari; Koivunen, Erkki; Kubo, Hajime (Ludwig Institute for Cancer Research, USA; Licentia Ltd.). PCT Int. Appl. WO 2002057299 A2 20020725, 149 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,

LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-IB99 20020116. PRIORITY: US 2001-2001/PV262476 20010117.

AB The present invention relates to the diagnostics, evaluation, and therapeutic intervention of disorders mediated by the activity of cell surface receptor VEGFR-3, which activity often is stimulated by VEGFR-3 ligands VEGF-C and VEGF-D. More particularly, the present invention identifies novel methods and compns. for the inhibition of VEGF-C/D binding to VEGFR-3. The compns. of the present invention will be useful the inhibition of angiogenesis and lymphangiogenesis. Many uses of such compds., for screening samples, imaging, diagnosis, and therapy, are also provided. For example, in one embodiment, the invention provides an isolated peptide comprising the formula: X1X2X3X4X5X6X7X8, wherein X1 through X8 are amino acid residues.

=> s GYWLTIWG

L22 0 GYWLTIWG

=> s cyclic peptide

L23 13602 CYCLIC PEPTIDE

=> s l23 and 8-100 amino acids

L24 0 L23 AND 8-100 AMINO ACIDS

=> s l23 and VEGF-C

L25 2 L23 AND VEGF-C

=> dup remove l25

PROCESSING COMPLETED FOR L25

L26 2 DUP REMOVE L25 (0 DUPLICATES REMOVED)

=> d l26 1-2 cbib abs

L26 ANSWER 1 OF 2 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

2005:903474 The Genuine Article (R) Number: 960XF. Modulating furin activity with designed mini-PDX peptides: Synthesis and in vitro kinetic evaluation . Basak A (Reprint); Lotfipour F. Univ Ottawa, Ottawa Hlth Res Inst, Dis Aging Program, Reg Prot Chem Ctr, Loeb Bldg, 725 Parkdale Ave, Ottawa, ON K1Y 4E9, Canada (Reprint); Univ Ottawa, Ottawa Hlth Res Inst, Dis Aging Program, Reg Prot Chem Ctr, Ottawa, ON K1Y 4E9, Canada; Tabriz Univ Med Sci, Fac Pharm, Dept Pharmaceut, Tabriz, Iran. abasak@ohri.ca. FEBS LETTERS (29 AUG 2005) Vol. 579, No. 21, pp. 4813-4821. ISSN: 0014-5793. Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A peptide was designed from reactive site loop structure of alpha 1 Antitrypsin Portland known as alpha 1 PDX as a novel mini-PDX inhibitor of furin. The sequence was derived from (367-394) that contains the crucial furin cleavage motif R1PR382. A P3 mutant replacing Ile(380) by Leu was prepared as a first model peptide. A Cys residue was inserted at each terminal of the peptide for purpose of cyclisation which was accomplished by air or iodine-induced oxidation. This mini-PDX peptide both cyclic and acyclic form inhibited in vitro furin activity (IC50 in nM) when measured against either substrates Boc-RVRR double down arrow MCA or QVEGF-C vertical bar Abz-QVHSIIRR double down arrow SLP-Y(NO2)-A-CONH2, Abz = 2-amino benzoic acid and Y(NO2) = 3-nitro tyrosine vertical bar, latter being derived from vascular endothelial growth factor-C (VEGF-C) processing site. The geometrically constrained structure

mimicking PDX reactive loop is crucial for enzyme inhibition. Our study further revealed that both mini-PDX peptides inactivate furin in a slow tight binding manner, with disulfide-bridged cyclic form being slightly more potent. Unlike PDX, these peptides inhibit furin via a different mechanistic pathway. The study provides an alternate strategy for development of efficient peptide-based inhibitors of Proprotein Convertases including furin.

L26 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2006 ACS on STN

2001:545508 Document No. 135:132464 **Cyclic peptide** inhibitors of VEGF, **VEGF-C**, and VEGF-D, preparation methods, pharmaceutical compositions, and therapeutic use. Achen, Marc G.; Hughes, Richard A.; Stacker, Steven; Cendron, Angela (Ludwig Institute for Cancer Research, USA). PCT Int. Appl. WO 2001052875 A1 20010726, 102 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US1533 20010118. PRIORITY: US 2000-PV176293 20000118; US 2000-PV204590 20000516.

AB The invention provides monomeric monocyclic peptide inhibitors and dimeric bicyclic peptide inhibitors based on exposed loop fragments of a growth factor protein, e.g. loop 1, loop 2 or loop 3 of VEGF-D, as well as methods of making them, pharmaceutical compns. containing them, and therapeutic methods of use.

=> s (alitalo k?/au or koivunen e?/au kubo h?/au)
'?' TRUNCATION SYMBOL NOT VALID WITHIN 'KOIVUNEN E?/AU KUBO H?'
The truncation symbol ? may be used only at the end of a search term. To specify a variable character within a word use '!', e.g., 'wom!n' to search for both 'woman' and 'women'. Enter "HELP TRUNCATION" at an arrow prompt (=>) for more information.

=> s (alitalo k?/au or koivunen e?/au or Kubo h?/au)
L27 7897 (ALITALO K?/AU OR KOIVUNEN E?/AU OR KUBO H?/AU)

=> s l27 and VEGF-C
L28 547 L27 AND VEGF-C

=> s l28 and derivative
L29 10 L28 AND DERIVATIVE

=> dup remove l29
PROCESSING COMPLETED FOR L29
L30 6 DUP REMOVE L29 (4 DUPLICATES REMOVED)

=> d l30 1-6 cbib abs

L30 ANSWER 1 OF 6 CAPLUS COPYRIGHT 2006 ACS on STN

2005:122784 Document No. 142:212747 Fusion proteins of vascular endothelial growth factor homology domains and heparin-binding domains for use as ligands of vascular endothelial growth factor receptor 3. **Alitalo, Kari**; He, Yulong; Tammela, Toumas (Finland). U.S. Pat. Appl. Publ. US 2005032697 A1 20050210, 119 pp., Cont.-in-part of U.S. Ser. No. 669,176. (English). CODEN: USXXCO. APPLICATION: US 2004-868577 20040614. PRIORITY: US 2003-2003/PV47811U 20030612; US 2003-2003/PV47839U 20030612; US 2003-2003/669176 20030923.

AB Synthetic ligands for vascular endothelial growth factor receptor 3 that

are fusion proteins of a heparin-binding domain and a vascular endothelial growth factor homol. domain and that show greater heparin-binding are described for use in therapeutic control of angiogenesis. These synthetic ligands show greater binding to heparin and the VEGFR3 receptor than its native ligands, **VEGF-C** and VEGF-D. A fusion protein of **VEGF-C** and the heparin-binding domain of vascular endothelial growth factor was manufactured by expression of the corresponding gene in 293T or 293EBNA cells. The proteins had greater lymphangiogenic activity than **VEGF-C** in guinea pigs, although the induced blood vessels were leaky.

L30 ANSWER 2 OF 6 CAPLUS COPYRIGHT 2006 ACS on STN

2003:293770 Document No. 139:240734 Vascular endothelial growth factor (VEGF) receptor-2 signaling mediates **VEGF-C**

Δ NAC- and VEGF-A-induced angiogenesis in vitro. Tille, Jean-Christophe; Wang, Xueyan; Lipson, Kenneth E.; McMahon, Gerald; Ferrara, Napoleone; Zhu, Zhenping; Hicklin, Daniel J.; Sleeman, Jonathan P.; Eriksson, Ulf; **Alitalo, Kari**; Pepper, Michael S. (Department of Cell Biology and Morphology, University Medical Center, Geneva, Switz.). Experimental Cell Research, 285(2), 286-298 (English) 2003. CODEN: ECREAL. ISSN: 0014-4827. Publisher: Elsevier Science.

AB Angiogenesis and lymphangiogenesis are regulated by members of the vascular endothelial growth factor (VEGF) family of cytokines, which mediate their effects via tyrosine kinase VEGF receptors -1, -2, and -3. The authors have used wild-type and mutant forms of VEGFs -A, -B, and -C, a pan-VEGFR tyrosine kinase inhibitor (SU5416) as well as neutralizing anti-VEGFR-2 antibodies, to determine which VEGF receptor(s) are required for bovine endothelial cell invasion and tube formation in vitro. This was compared to the ability of these cytokines to induce expression of members of the plasminogen activator (PA)-plasmin system. The authors found that cytokines which bind VEGFR-2 (human VEGF-A, human VFM23A, human **VEGF-C.DELTA.NAC**, and rat VEGF-C152) induced invasion, tube formation, urokinase-type-PA, tissue-type-PA, and PA inhibitor-1, invasion and tube formation as well as signaling via the MAP kinase pathway were efficiently blocked by SU5416 and anti-VEGFR-2 antibodies. In contrast, cytokines and mutants which exclusively bind VEGFR-1 (human VFM17 and human VEGF-B) had no effect on invasion and tube formation or on the regulation of gene expression. The authors were unable to identify cytokines which selectively stimulate bovine VEGFR-3 in the authors' system. Taken together, these findings point to the central role of VEGFR-2 in the angiogenic signaling pathways induced by **VEGF-C.DELTA.NAC** and VEGF-A.

L30 ANSWER 3 OF 6 CAPLUS COPYRIGHT 2006 ACS on STN

2002:555522 Document No. 137:119669 VEGFR-3 inhibitor materials and methods.

Alitalo, Kari; Koivunen, Erkki; Kubo, Hajime

(Ludwig Institute for Cancer Research, USA; Licentia Ltd.). PCT Int. Appl. WO 2002057299 A2 20020725, 149 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-IB99 20020116. PRIORITY: US 2001-2001/PV262476 20010117.

AB The present invention relates to the diagnostics, evaluation, and therapeutic intervention of disorders mediated by the activity of cell surface receptor VEGFR-3, which activity often is stimulated by VEGFR-3 ligands **VEGF-C** and VEGF-D. More particularly, the present invention identifies novel methods and compns. for the inhibition of **VEGF-C/D** binding to VEGFR-3. The compns. of the

present invention will be useful the inhibition of angiogenesis and lymphangiogenesis. Many uses of such compds., for screening samples, imaging, diagnosis, and therapy, are also provided. For example, in one embodiment, the invention provides an isolated peptide comprising the formula: X1X2X3X4X5X6X7X8, wherein X1 through X8 are amino acid residues.

L30 ANSWER 4 OF 6 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN

2002440932 EMBASE Lymphatic endothelial regulation, lymphoedema, and lymph node metastasis. Karkkainen M.J.; **Alitalo K.** M.J. Karkkainen, Molecular/Cancer Biology Laboratory, Helsinki University Hospital, University of Helsinki, PO Box 63 (Haartmaninkatu 8), 00014 Helsinki, Finland. Marika.Karkkainen@Helsinki.Fi. Seminars in Cell and Developmental Biology Vol. 13, No. 1, pp. 9-18 2002.

Refs: 100.

ISSN: 1084-9521. CODEN: SCDBFX

Pub. Country: United Kingdom. Language: English. Summary Language: English.

Entered STN: 20021219. Last Updated on STN: 20021219

AB Vascular endothelial growth factor receptor-3 (VEGFR-3) mediates lymphatic endothelial cell (LEC) growth, migration, and survival by binding **VEGF-C** and VEGF-D. Recent studies have revealed new regulators of the lymphatic endothelium, such as the transcription factor Prox1, and the cell surface proteins podoplanin and lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1). Furthermore, the isolation of LECs now allows detailed molecular studies of the factors regulating the lymphatic vasculature. These studies are aimed at targeting the lymphatic vasculature in the treatment of various diseases, such as tumour metastasis and lymphoedema.

L30 ANSWER 5 OF 6 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

2001:945005 The Genuine Article (R) Number: 494QQ. Multiple forms of mouse vascular endothelial growth factor-D are generated by RNA splicing and proteolysis. Baldwin M E; Roufail S; Halford M M; **Alitalo K**; Stacker S A; Achen M G (Reprint). Royal Melbourne Hosp, Ludwig Inst Canc Res, POB 2008, Melbourne, Vic 3050, Australia (Reprint); Royal Melbourne Hosp, Ludwig Inst Canc Res, Melbourne, Vic 3050, Australia; Univ Helsinki, Mol Canc Biol Lab, FIN-00014 Helsinki, Finland. JOURNAL OF BIOLOGICAL CHEMISTRY (23 NOV 2001) Vol. 276, No. 47, pp. 44307-44314. ISSN: 0021-9258. Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The secreted glycoprotein vascular endothelial growth factor-D (VEGF-D) is angiogenic, lymphangiogenic, and promotes metastatic spread of tumor cells via lymphatic vessels. VEGF-D consists of a receptor-binding domain (VEGF homology domain) and N- and G terminal propeptides. Proteolytic processing produces numerous forms of human VEGF-D, including fully processed **derivatives** (containing only the VEGF homology domain), partially processed, and unprocessed **derivatives**. Proteolysis is essential to generate human VEGF-D that binds the angiogenic receptor VEGF receptor-2 (VEGFR-2) and the lymphangiogenic receptor VEGFR-3 with high affinity. Here, we report that alternative use of an RNA splice donor site in exon 6 of the mouse VEGF-D gene produces two different protein isoforms, VEGF-D-358 and VEGF-D-326, with distinct C termini. The two isoforms were both expressed in all adult mouse tissues and embryonic stages of development analyzed. Both isoforms are proteolytically processed in a similar fashion to human VEGF-D to generate a range of secreted **derivatives** and bind and cross-link VEGFR-3 with similar potency. The isoforms are differently glycosylated when expressed in vitro. This study demonstrates that RNA splicing, protein glycosylation, and proteolysis are mechanisms for generating structural diversity of mouse VEGF-D.

L30 ANSWER 6 OF 6 MEDLINE on STN DUPLICATE 1
 1998118549. PubMed ID: 9435229. Vascular endothelial growth factor D (VEGF-D) is a ligand for the tyrosine kinases VEGF receptor 2 (Flk1) and VEGF receptor 3 (Flt4). Achen M G; Jeltsch M; Kukk E; Makinen T; Vitali A; Wilks A F; **Alitalo K**; Stacker S A. (Ludwig Institute for Cancer Research, Royal Melbourne Hospital, Victoria, Australia.. Marc.achen@ludwig.edu.au) . Proceedings of the National Academy of Sciences of the United States of America, (1998 Jan 20) Vol. 95, No. 2, pp. 548-53. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB We have identified a member of the VEGF family by computer-based homology searching and have designated it VEGF-D. VEGF-D is most closely related to **VEGF-C** by virtue of the presence of N- and C-terminal extensions that are not found in other VEGF family members. In adult human tissues, VEGF-D mRNA is most abundant in heart, lung, skeletal muscle, colon, and small intestine. Analyses of VEGF-D receptor specificity revealed that VEGF-D is a ligand for both VEGF receptors (VEGFRs) VEGFR-2 (Flk1) and VEGFR-3 (Flt4) and can activate these receptors. However. VEGF-D does not bind to VEGFR-1. Expression of a truncated **derivative** of VEGF-D demonstrated that the receptor-binding capacities reside in the portion of the molecule that is most closely related in primary structure to other VEGF family members and that corresponds to the mature form of **VEGF-C**. In addition, VEGF-D is a mitogen for endothelial cells. The structural and functional similarities between VEGF-D and **VEGF-C** define a subfamily of the VEGFs.

=> s 128 and VEGFR-3

L31 352 L28 AND VEGFR-3

=> dup remove 131

PROCESSING COMPLETED FOR L31

L32 121 DUP REMOVE L31 (231 DUPLICATES REMOVED)

=> d 132 1-121 cbib abs

L32 ANSWER 1 OF 121 CAPLUS COPYRIGHT 2006 ACS on STN
 2006:373443 Vascular Endothelial Growth Factor (VEGF)/**VEGF-C** Mosaic Molecules Reveal Specificity Determinants and Feature Novel Receptor Binding Patterns. Jeltsch, Michael; Karpanen, Terhi; Strandin, Tomas; Aho, Kukka; Lankinen, Hilka; **Alitalo, Kari** (Molecular/Cancer Biology Laboratory and Ludwig Institute for Cancer Research, Biomedicum Helsinki, Haartman Institute and Helsinki University Central Hospital, P.O. Box 63 (Haartmaninkatu 8), University of Helsinki and Peptide and Protein Laboratory, Department of Virology, Finland). Journal of Biological Chemistry, 281(17), 12187-12195 (English) 2006. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB Vascular endothelial growth factors (VEGFs) and their receptors play key roles in angiogenesis and lymphangiogenesis. VEGF activates VEGF receptor-1 (VEGFR-1) and VEGFR-2, whereas **VEGF-C** activates VEGFR-2 and **VEGFR-3**. We have created a library of VEGF/**VEGF-C** mosaic mols. that contains factors with novel receptor binding profiles, notably proteins binding to all three VEGF receptors ("super-VEGFs"). The analyzed super-VEGFs show both angiogenic and lymphangiogenic effects in vivo, although weaker than the parental mols. The composition of the **VEGFR-3** binding mols. and scanning mutagenesis revealed determinants of receptor binding and specificity. VEGFR-2 and **VEGFR-3** showed striking differences in their requirements for **VEGF-C** binding; extracellular domain 2 of VEGFR-2 was sufficient, whereas in **VEGFR**

-3, both domains 1 and 2 were necessary.

L32 ANSWER 2 OF 121 MEDLINE on STN DUPLICATE 1

2006158479. PubMed ID: 16462734. **VEGF-C** is a trophic factor for neural progenitors in the vertebrate embryonic brain. Le Bras Barbara; Barallobre Maria-Jose; Homman-Ludiye Jihane; Ny Annelii; Wyns Sabine; Tammela Tuomas; Haiko Paula; Karkkainen Marika J; Yuan Li; Muriel Marie-Paule; Chatzopoulou Elli; Breant Christiane; Zalc Bernard; Carmeliet Peter; **Alitalo Kari**; Eichmann Anne; Thomas Jean-Leon. (Institut National de la Sante et de la Recherche Medicale (INSERM), U711, Paris F-75013, France.) Nature neuroscience, (2006 Mar) Vol. 9, No. 3, pp. 340-8. Electronic Publication: 2006-02-05. Journal code: 9809671. ISSN: 1097-6256. Pub. country: United States. Language: English.

AB Vascular endothelial growth factor C (**VEGF-C**) was first identified as a regulator of the vascular system, where it is required for the development of lymphatic vessels. Here we report actions of **VEGF-C** in the central nervous system. We detected the expression of the **VEGF-C** receptor **VEGFR-3** in neural progenitor cells in *Xenopus laevis* and mouse embryos. In *Xenopus* tadpole **VEGF-C** knockdowns and in mice lacking *Vegfc*, the proliferation of neural progenitors expressing **VEGFR-3** was severely reduced, in the absence of intracerebral blood vessel defects. In addition, *Vegfc*-deficient mouse embryos showed a selective loss of oligodendrocyte precursor cells (OPCs) in the embryonic optic nerve. In vitro, **VEGF-C** stimulated the proliferation of OPCs expressing **VEGFR-3** and nestin-positive ventricular neural cells. **VEGF-C** thus has a new, evolutionary conserved function as a growth factor selectively required by neural progenitor cells expressing its receptor **VEGFR-3**.

L32 ANSWER 3 OF 121 CAPLUS COPYRIGHT 2006 ACS on STN

2005:1027025 Document No. 143:324148 Signal transduction pathways in lymphatic system differentiation and the therapeutic control of lymphatic and venous arterIALIZATION. **Alitalo, Kari**; Petrova, Tatiana; Karpanen, Terhi; Norrmén, Camilla (Ludwig Institute for Cancer Research, USA; Licentia, Ltd.). PCT Int. Appl. WO 2005087954 A2 20050922, 236 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IS, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2005-US7693 20050308. PRIORITY: US 2004-2004/PV551581 20040308.

AB The interactions of genes and gene products playing a major role in the normal development of the lymphatic system is analyzed to identify possible targets for the treatment of abnormal development of the lymphatic and vascular systems. In particular, treatments are sought for lymphedema distichiasis and chronic venous insufficiency. The interactions of the forkhead box transcription factor **FOXC2** and vascular endothelial growth factor receptor 3 are analyzed in mouse.

L32 ANSWER 4 OF 121 CAPLUS COPYRIGHT 2006 ACS on STN

2005:1026969 Document No. 143:324792 Multivalent antibodies specific to growth factors of VEGF/PDGF family for diagnosis and treatment of fibrosis, inflammation, cancer and other diseases associated with aberrant angiogenesis. Eriksson, Ulf; **Alitalo, Kari**; Achen, Marc G.; Renner, Christoph; Stacker, Stephen; Li, Hong; Laakkonen, Pirjo (Ludwig Institute for Cancer Research, USA; Licentia Ltd.). PCT Int. Appl. WO 2005087812 A1 20050922, 152 pp. DESIGNATED STATES: W: AE, AG, AL, AM,

AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IS, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2005-US7742 20050307. PRIORITY: US 2004-2004/PV55051U 20040305; US 2004-2004/PV586662 20040709.

AB The present invention relates to materials and methods for modulating angiogenesis. The compns. of the invention provide antibody substances specific for two or more PDGF/VEGF family members, which are useful for modulating angiogenesis and lymphangiogenesis in a subject.

L32 ANSWER 5 OF 121 CAPLUS COPYRIGHT 2006 ACS on STN

2005:1026967 Document No. 143:332468 Growth factor receptor fragments for use in antitumor therapy. **Alitalo, Kari**; Jeltsch, Markku M. (Ludwig Institute for Cancer Research, USA; Licentia, Ltd.). PCT Int. Appl. WO 2005087808 A2 20050922, 338 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IS, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2005-US7741 20050307. PRIORITY: US 2004-2004/PV550907 20040305.

AB The present invention provides materials and methods for antagonizing the function of vascular endothelial growth factor receptors, platelet derived growth factor receptors and other receptors. Soluble binding constructs able to bind vascular endothelial growth factors, platelet derived growth factors, and other ligands are provided. These constructs may be used in treatment of cancers of endothelium and smooth muscle tissues, e.g., carcinomas, squamous cell carcinomas, lymphomas, melanomas, and sarcomas. Thus, chimeric proteins comprising the ligand-binding extracellular domain, or fragments thereof, of VEGFR-2 and **VEGFR-3** fused to the Fc domain of human IgG were prepared with recombinant E. coli. These proteins bound to VEGF-A and/or **VEGF-C**.

L32 ANSWER 6 OF 121 CAPLUS COPYRIGHT 2006 ACS on STN

2005:300273 Document No. 142:349474 **VEGF-C** or VEGF-D materials and methods for stimulation of neural stem cells in therapeutic applications. **Alitalo, Kari**; Karkkainen, Marika; Haiko, Paula; Sainio, Kirsi; Wartiovaara, Kirmo; Thomas, Jean Leon; Eichmann, Anne (Ludwig Institute for Cancer Research, USA; Licentia Ltd.; Institut National de la Sante et de la Recherche Medicale INSERM). PCT Int. Appl. WO 2005030240 A2 20050407, 263 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2004-US31318 20040923. PRIORITY: US 2003-2003/PV50560U 20030923; US 2003-2003/669176 20030923.

AB The present invention relates to **VEGF-C** or VEGF-D materials and methods for promoting growth and differentiation of neural stem cells, neuronal and neuronal precursor cells, oligodendrocytes and oligodendrocyte precursor cells and materials and methods for administering said cells to inhibit neuropathol. The **VEGF-C** material comprises a purified mammalian prepro-**VEGF-C**

C polypeptide or fragment thereof that binds **VEGFR-3** or neuropilin-2 or polynucleotides encoding a **VEGF-C** product. Use of a **VEGF-C** inhibitor in the manufacture of a medicament for the treatment of a neuroblastoma or neural tumor is also claimed, as is a method for screening for modulators of **VEGF-C** stimulation of neural stem cell or neural precursor cell growth, migration, differentiation, or survival.

L32 ANSWER 7 OF 121 CAPLUS COPYRIGHT 2006 ACS on STN

2005:120758 Document No. 142:212746 Use of **VEGF-C** or **VEGF-D** in reconstructive surgery to reduce edema and improve skin perfusion. **Alitalo, Kari**; Saaristo, Anne; Karkkainen, Marika; Tammela, Tuomas; Asko-Seljavaara, Sirpa; Yla-Herttuala, Seppo; He, Yulong (Ludwig Institute for Cancer Research, USA; Licentia Ltd.). PCT Int. Appl. WO 2005011722 A2 20050210, 119 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2004-US19197 20040614. PRIORITY: US 2003-2003/PV47839U 20030612; US 2003-2003/PV478114 20030612.

AB The present invention provides materials and methods for repairing tissue and using vascular endothelial growth factor C (**VEGF-C**) genes and/or proteins. Methods and materials related to the use of **VEGF-C** for the reduction of edema and improvement of skin perfusion is provided. Also provided are materials and methods for using **VEGF-C** before, during, and after reconstructive surgery.

L32 ANSWER 8 OF 121 CAPLUS COPYRIGHT 2006 ACS on STN

2005:122784 Document No. 142:212747 Fusion proteins of vascular endothelial growth factor homology domains and heparin-binding domains for use as ligands of vascular endothelial growth factor receptor 3. **Alitalo, Kari**; He, Yulong; Tammela, Tuomas (Finland). U.S. Pat. Appl. Publ. US 2005032697 A1 20050210, 119 pp., Cont.-in-part of U.S. Ser. No. 669,176. (English). CODEN: USXXCO. APPLICATION: US 2004-868577 20040614. PRIORITY: US 2003-2003/PV47811U 20030612; US 2003-2003/PV47839U 20030612; US 2003-2003/669176 20030923.

AB Synthetic ligands for vascular endothelial growth factor receptor 3 that are fusion proteins of a heparin-binding domain and a vascular endothelial growth factor homol. domain and that show greater heparin-binding are described for use in therapeutic control of angiogenesis. These synthetic ligands show greater binding to heparin and the VEGFR3 receptor than its native ligands, **VEGF-C** and **VEGF-D**. A fusion protein of **VEGF-C** and the heparin-binding domain of vascular endothelial growth factor was manufactured by expression of the corresponding gene in 293T or 293EBNA cells. The proteins had greater lymphangiogenic activity than **VEGF-C** in guinea pigs, although the induced blood vessels were leaky.

L32 ANSWER 9 OF 121 MEDLINE on STN DUPLICATE 2

2005416286. PubMed ID: 16061674. Inhibition of lymphogenous metastasis using adeno-associated virus-mediated gene transfer of a soluble **VEGFR-3** decoy receptor. Lin JianMin; Lalani Alshad S; Harding Thomas C; Gonzalez Melissa; Wu Wei-Wei; Luan Bo; Tu Guang Huan; Koprivnikar Kathryn; VanRoey Melinda J; He Yulong; **Alitalo Kari**; Jooss Karin. (Department of Preclinical Oncology and Immunology, Cell Genesys, Inc., South San Francisco, California 94080, USA.. jianmin.lin@cellgenesys.com) . Cancer research, (2005 Aug 1) Vol. 65, No. 15, pp. 6901-9. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB The presence of metastases in regional lymph nodes is a strong indicator of poor patient survival in many types of cancer. It has recently been shown that the lymphangiogenic growth factor, vascular endothelial growth factor-C (**VEGF-C**), and its receptor, VEGF receptor-3 (VEGFR3), may play a pivotal role in the promotion of metastasis to regional lymph nodes. In this study, human prostate and melanoma tumor models that preferentially metastasize to the lymph nodes following s.c. tumor cell implantation were established from lymph node metastases via in vivo selection. Melanoma tumor cell sublines established from lymph node metastasis express higher amounts of **VEGF-C** than the parental tumor cells. The inhibition of tumor-derived **VEGF-C** with a soluble VEGFR3 decoy receptor, sVEGFR3-Fc, expressed via a recombinant adeno-associated viral vector, potently blocks tumor-associated lymphangiogenesis and tumor metastasis to the lymph nodes, when the treatment was initiated before the tumor implantation. In addition, sVEGFR3-Fc serum levels required for efficient blockade of lymph node metastases are strictly dependent on the **VEGF-C** levels generated by the primary tumor. Recombinant adeno-associated virus-mediated gene transfer of sVEGFR3-Fc may represent a feasible therapeutic strategy for blockade of lymphogenous metastasis.

L32 ANSWER 10 OF 121 MEDLINE on STN DUPLICATE 3
 2005285073. PubMed ID: 15930292. Vascular endothelial cell growth factor receptor 3-mediated activation of lymphatic endothelium is crucial for tumor cell entry and spread via lymphatic vessels. He Yulong; Rajantie Iiro; Pajusola Katri; Jeltsch Michael; Holopainen Tanja; Yla-Herttuala Seppo; Harding Thomas; Jooss Karin; Takahashi Takashi; **Alitalo Kari**. (Molecular/Cancer Biology Laboratory and Ludwig Institute for Cancer Research, Biomedicum Helsinki and Helsinki University Central Hospital, University of Helsinki, Helsinki, Finland.) Cancer research, (2005 Jun 1) Vol. 65, No. 11, pp. 4739-46. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB Lymphangiogenic growth factors vascular endothelial growth factor (**VEGF**)-C and VEGF-D have been shown to promote lymphatic metastasis by inducing tumor-associated lymphangiogenesis. In this study, we have investigated how tumor cells gain access into lymphatic vessels and at what stage tumor cells initiate metastasis. We show that **VEGF-C** produced by tumor cells induced extensive lymphatic sprouting towards the tumor cells as well as dilation of the draining lymphatic vessels, suggesting an active role of lymphatic endothelial cells in lymphatic metastasis. A significant increase in lymphatic vessel growth occurred between 2 and 3 weeks after tumor xenotransplantation, and lymph node metastasis occurred at the same stage. These processes were blocked dose-dependently by inhibition of VEGF receptor 3 (**VEGFR-3**) signaling by systemic delivery of a soluble **VEGFR-3**-immunoglobulin (Ig) fusion protein via adenoviral or adeno-associated viral vectors. However, **VEGFR-3**-Ig did not suppress lymph node metastasis when the treatment was started at a later stage after the tumor cells had already spread out, suggesting that tumor cell entry into lymphatic vessels is a key step during tumor dissemination via the lymphatics. Whereas lymphangiogenesis and lymph node metastasis were significantly inhibited by **VEGFR-3**-Ig, some tumor cells were still detected in the lymph nodes in some of the treated mice. This indicates that complete blockade of lymphatic metastasis may require the targeting of both tumor lymphangiogenesis and tumor cell invasion.

L32 ANSWER 11 OF 121 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN
 2005:628920 The Genuine Article (R) Number: 935CQ. Angiopoietin-1 promotes lymphatic sprouting and hyperplasia. Tammela T; Saaristo A; Lohela M; Morisada T; Tomberg J; Norrmén C; Oike Y; Pajusola K; Thurston G; Suda T; Yla-Herttuala S; **Alitalo K (Reprint)**. Univ Helsinki, Mol Canc

Biol Lab, Biomedicum Helsinki, POB 63, Haartmaninkatu 8, FIN-00014 Helsinki, Finland (Reprint); Univ Helsinki, Mol Canc Biol Lab, Biomedicum Helsinki, FIN-00014 Helsinki, Finland; Univ Helsinki, Ludwig Inst Canc Res, Biomedicum Helsinki, FIN-00014 Helsinki, Finland; Univ Helsinki, Cent Hosp, FIN-00014 Helsinki, Finland; Univ Kuopio, AI Virtanen Inst, FIN-70211 Kuopio, Finland; Univ Kuopio, Dept Med, FIN-70211 Kuopio, Finland; Keio Univ, Sch Med, Sakaguchi Lab, Dept Cell Differentiat, Tokyo 108, Japan; Regeneron Pharmaceut Inc, Tarrytown, NY 10591 USA. kari.alitalo@helsinki.fi. BLOOD (15 JUN 2005) Vol. 105, No. 12, pp. 4642-4648. ISSN: 0006-4971. Publisher: AMER SOC HEMATOLOGY, 1900 M STREET. NW SUITE 200, WASHINGTON, DC 20036 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Angiopoietin 1 (Ang1), a ligand for the receptor tyrosine kinase Tie2, regulates the formation and stabilization of the blood vessel network during embryogenesis. In adults, Ang1 is associated with blood vessel stabilization and recruitment of perivascular cells, whereas Ang2 acts to counter these actions. Recent results from gene-targeted mice have shown that Ang2 is also essential for the proper patterning of lymphatic vessels and that Ang1 can be substituted for this function. In order to characterize the effects of the angiopoietins on lymphatic vessels, we employed viral vectors for overexpression of Ang1 in adult mouse tissues. We found that Ang1 activated lymphatic vessel endothelial proliferation, vessel enlargement, and generation of long endothelial cell filopodia that eventually fused, leading to new sprouts and vessel development. Cutaneous lymphatic hyperplasia was also detected in transgenic mice expressing Ang1 in the basal epidermal cells. Tie2 was expressed in the lymphatic endothelial cells and Ang1 stimulation of these cells resulted in up-regulation of vascular endothelial growth factor receptor 3 (**VEGFR-3**). Furthermore, a soluble form of **VEGFR-3** inhibited the observed lymphatic sprouting. Our results reinforce the concept that Ang1 therapy may be useful in settings of tissue edema. (c) 2005 by The American Society of Hematology.

L32 ANSWER 12 OF 121 MEDLINE on STN DUPLICATE 4
2005113639. PubMed ID: 15743836. Vascular endothelial growth factor D is dispensable for development of the lymphatic system. Baldwin Megan E; Halford Michael M; Roufail Sally; Williams Richard A; Hibbs Margaret L; Grail Dianne; **Kubo Hajime**; Stacker Steven A; Achen Marc G. (Ludwig Institute for Cancer Research, Royal Melbourne Hospital, PO Box 2008, Parkville, Victoria 3050, Australia.) Molecular and cellular biology, (2005 Mar) Vol. 25, No. 6, pp. 2441-9. Journal code: 8109087. ISSN: 0270-7306. Pub. country: United States. Language: English.

AB Vascular endothelial growth factor receptor 3 (**Vegfr-3**) is a tyrosine kinase that is expressed on the lymphatic endothelium and that signals for the growth of the lymphatic vessels (lymphangiogenesis). Vegf-d, a secreted glycoprotein, is one of two known activating ligands for **Vegfr-3**, the other being **Vegf-c**. Vegf-d stimulates lymphangiogenesis in tissues and tumors; however, its role in embryonic development was previously unknown. Here we report the generation and analysis of mutant mice deficient for Vegf-d. Vegf-d-deficient mice were healthy and fertile, had normal body mass, and displayed no pathologic changes consistent with a defect in lymphatic function. The lungs, sites of strong Vegf-d gene expression during embryogenesis in wild-type mice, were normal in Vegf-d-deficient mice with respect to tissue mass and morphology, except that the abundance of the lymphatics adjacent to bronchioles was slightly reduced. Dye uptake experiments indicated that large lymphatics under the skin were present in normal locations and were functional. Smaller dermal lymphatics were similar in number, location, and function to those in wild-type controls. The lack of a profound lymphatic phenotype in Vegf-d-deficient mice suggests that Vegf-d does not play a major role in lymphatic development or that **Vegf-c** or another, as-yet-unknown activating **Vegfr-3** ligand can compensate for Vegf-d during

development.

L32 ANSWER 13 OF 121 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

2005:145220 Document No.: PREV200500147279. The biology of vascular endothelial growth factors. Tammela, Tuornas; Enholm, Bemdt; **Alitalo, Karl**; Paavonen, Karri [Reprint Author]. Mol Canc Biol LabBiomed Helsinki, Univ Helsinki, POB 63 Haartmaninkatu 8, FIN-00014, Helsinki, Finland. karri.paavonen@helsinki.fi. Cardiovascular Research, (February 15 2005) Vol. 65, No. 3, pp. 550-563. print.
CODEN: CVREAU. ISSN: 0008-6363. Language: English.

AB The discovery of the vascular endothelial growth factor (VEGF) family members VEGF, VEGF-beta, placental growth factor (PIGF), **VEGF-C** and VEGF-D and their receptors VEGFR-1, -2 and -3 has provided tools for studying the vascular system in development as well as in diseases ranging from ischemic heart disease to cancer. VEGF has been established as the prime angiogenic molecule during development, adult physiology and pathology. PIGF may primarily mediate arteriogenesis, the formation of collateral arteries from preexisting arterioles, with potential future therapeutic use in for example occlusive atherosclerotic disease. **VEGF-C** and VEGF-D are primarily lymphangiogenic factors, but they can also induce angiogenesis in some conditions. While many studies have addressed the role of angiogenesis and the blood vasculature in human physiology, the lymphatic vascular system has until recently attracted very little attention. In this review, we will discuss recent advances in angiogenesis research and provide an overview of the molecular players involved in lymphangiogenesis. Copyright 2004 European Society of Cardiology. Published by Elsevier B.V. All rights reserved.

L32 ANSWER 14 OF 121 MEDLINE on STN DUPLICATE 5

2005075177. PubMed ID: 15668734. Pathogenesis of persistent lymphatic vessel hyperplasia in chronic airway inflammation. Baluk Peter; Tammela Tuomas; Ator Erin; Lyubynska Natalya; Achen Marc G; Hicklin Daniel J; Jeltsch Michael; Petrova Tatiana V; Pytowski Bronislaw; Stacker Steven A; Yla-Herttuala Seppo; Jackson David G; **Alitalo Kari**; McDonald Donald M. (Cardiovascular Research Institute, Comprehensive Cancer Center, and Department of Anatomy, UCSF, San Francisco, California 94143, USA.) The Journal of clinical investigation, (2005 Feb) Vol. 115, No. 2, pp. 247-57. Journal code: 7802877. ISSN: 0021-9738. Pub. country: United States. Language: English.

AB Edema occurs in asthma and other inflammatory diseases when the rate of plasma leakage from blood vessels exceeds the drainage through lymphatic vessels and other routes. It is unclear to what extent lymphatic vessels grow to compensate for increased leakage during inflammation and what drives the lymphangiogenesis that does occur. We addressed these issues in mouse models of (a) chronic respiratory tract infection with Mycoplasma pulmonis and (b) adenoviral transduction of airway epithelium with VEGF family growth factors. Blood vessel remodeling and lymphangiogenesis were both robust in infected airways. Inhibition of **VEGFR-3** signaling completely prevented the growth of lymphatic vessels but not blood vessels. Lack of lymphatic growth exaggerated mucosal edema and reduced the hypertrophy of draining lymph nodes. Airway dendritic cells, macrophages, neutrophils, and epithelial cells expressed the **VEGFR-3** ligands **VEGF-C** or VEGF-D. Adenoviral delivery of either **VEGF-C** or VEGF-D evoked lymphangiogenesis without angiogenesis, whereas adenoviral VEGF had the opposite effect. After antibiotic treatment of the infection, inflammation and remodeling of blood vessels quickly subsided, but lymphatic vessels persisted. Together, these findings suggest that when lymphangiogenesis is impaired, airway inflammation may lead to bronchial lymphedema and exaggerated airflow obstruction. Correction of defective lymphangiogenesis may benefit the treatment of asthma and other

inflammatory airway diseases.

L32 ANSWER 15 OF 121 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on
STN DUPLICATE 6

2006:208237 Document No.: PREV200600209965. Enhanced **VEGFR-3**
and Lyve-1 expression despite decreased **VEGF-C** and
VEGF-D expression in duodenal mucosa of idiopathic lymphangiectasia with
enteric protein-loss. Hokari, Ryota; Kitagawa, Noritake; Tsuzuki,
Yoshikazu; Kato, Shingo; Kawaguchi, Atsushi; Nagao, Shigeaki; Kurihara,
Chic; Okada, Yoshikiyo; **Alitalo, Kari**; Miura, Soichiro.
Gastroenterology, (APR 2005) Vol. 128, No. 4, Suppl. 2, pp. A188.
Meeting Info.: Annual Meeting of the American-Gastroenterological-
Association/Digestive-Disease-Week. Chicago, IL, USA. May 14 -19, 2005.
Amer Gastroenterol Assoc.

CODEN: GASTAB. ISSN: 0016-5085. Language: English.

AB Background: Vascular endothelial growth factor receptor-3 (**VEGFR-3**), a receptor protein tyrosine kinase and LYVE-1, a hyaluronan receptor, are specifically expressed in the adult lymphatic endothelium. On the other hand **VEGF-C** and D, ligands for **VEGFR-3**, induce selective hyperplasia of the lymphatic vasculature and are known to play central roles in lymphangiogenesis. In this study, we investigated the expression of **VEGFR-3**, LYVE-I and **VEGF-C/D** in the duodenal mucosa of idiopathic intestinal lymphangiectasia. Methods: Tissue samples were obtained from duodenal biopsies in patients With idiopathic intestinal lymphangiectasia complicated with protein-losing with informed consent, Biospies were taken from white spot lesions. As controls biopsy specimens were obtained from healthy subjects. For immunohistochemical analysis, antibodies against human VEGFR3 and LYVF-1 were used as lymphatic markers. Anti-endothelium (PAL-E) and anti-CD34 antibodies were used to identify venular endothelium. Messenger RNA expression of **VEGF-C**, VEGF-D, and **VEGFR-3** in the mucosa was determined by quantitative PCR method. mRNA expressions of other markers for lymphatic vessel development, Prox 1 and FOXC2 were also examined, Results: In the control mucosa VEGFR3 was only weakly expressed on the central lymphatic vessels in the lamina propria of intestine and LYVE-1 was expressed mainly on the lymphatic vessels in the submucosa. PAL-E and CD34 were expressed within the venules just below the epithelial cell layer of duodenal villi. On the other hand in the mucosa of intestinal lymphangiectasia, VEGFR3 and LYVE-1 expression was increased and the intense expression site appeared to correspond to the widely dilated central lymphatic vessels. Messenger RNA expression study showed a significant increase in VEGFR3 in lymphangiectasia. However, the expression of **VEGF-C** and VEGF-D mRNA were significantly suppressed despite of the presence of lymphangiectasia compared with controls. mRNA expression of other markers for lymph development, Prox 1 and FOXC2 were also decreased. Conclusions: The present results suggest that there is an increased growth of lymphatic endothelial cells through **VEGFR-3** and LYVE-1 receptors in dilated central lymphatic vessels of idiopathic lymphangiectasia, although expressions of lymphangiogenic growth factors are decreased. There is a possibility that there is a dysregulation of lymphangiogenesis which may be closely related to the pathophysiology of this disease.

L32 ANSWER 16 OF 121 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on
STN

2004:469230 Document No.: PREV200400474157. Vascular endothelial growth factor C (**VEGF-C**) protein and gene mutants thereof, and uses thereof. **Alitalo, Kari** [Inventor, Reprint Author]; Joukov, Vladimir [Inventor]. Helsinki, Finland. ASSIGNEE: Licentia Ltd., Helsinki, Finland; Ludwig Institute for Cancer Research. Patent Info.: US 6818220 20041116. Official Gazette of the United States Patent and Trademark Office Patents, (Nov 16 2004) Vol. 1288, No. 3.
<http://www.uspto.gov/web/menu/patdata.html>. e-file.

ISSN: 0098-1133 (ISSN print). Language: English.

- AB Provided are purified and isolated **VEGF-C** polypeptides capable of binding to at least one of KDR receptor tyrosine kinase (VEGFR-2) and Flt4 receptor tyrosine kinase (**VEGFR-3**); analogs of such peptides that have **VEGF-C**-like or VEGF-like biological activities or that are VEGF or **VEGF-C** inhibitors; polynucleotides encoding the polypeptides; vectors and host cells that embody the polynucleotides; pharmaceutical compositions and diagnostic reagents comprising the polypeptides; and methods of making and using the polypeptides.

L32 ANSWER 17 OF 121 CAPLUS COPYRIGHT 2006 ACS on STN

2004:905599 Document No. 141:375099 Methods for the use of **VEGF-C** or VEGF-D products for the treatment of neuropathologies. **Alitalo, Kari**; Karkkainen, Marika; Haiko, Paula; Sainio, Kirsi; Wartiovaara, Kirmo (Finland). U.S. Pat. Appl. Publ. US 2004214766 A1 20041028, 125 pp., Cont.-in-part of U.S. Ser. No. 262,538. (English). CODEN: USXXCO. APPLICATION: US 2003-669176 20030923. PRIORITY: US 2001-2001/PV32632U 20011001; US 2002-2002/262538 20020930.

- AB The present invention relates to **VEGF-C** or VEGF-D materials and methods for promoting growth and differentiation of neural stem cells and materials and methods for administering said cells to inhibit neuropathol.

L32 ANSWER 18 OF 121 CAPLUS COPYRIGHT 2006 ACS on STN

2004:510772 Document No. 141:66280 Modulation of vascular endothelial growth factor C (**VEGF-C**)/vascular endothelial growth factor receptor **VEGFR-3** interactions using **VEGFR-3** inhibitor for treatment of rheumatoid arthritis. **Alitalo, Kari**; Paavonen, Karri; Konttinen, Yrjo (Finland). U.S. Pat. Appl. Publ. US 2004120950 A1 20040624, 32 pp. (English). CODEN: USXXCO. APPLICATION: US 2002-326048 20021220.

- AB The present invention provides methods for treating human chronic arthritides such as rheumatoid arthritis by modulation vascular endothelial growth factor C (**VEGF-C**)/vascular endothelial growth factor receptor **VEGFR-3** interactions. **VEGFR-3** inhibitors such as anti-**VEGFR-3** antibody, short-interfering RNA (siRNA), or **VEGFR-3** extracellular domain-containing polypeptides are administered at synovial sites.

L32 ANSWER 19 OF 121 CAPLUS COPYRIGHT 2006 ACS on STN

2004:1040136 Document No. 142:21235 Loss of neural cell adhesion molecule induces tumor metastasis by up-regulating lymphangiogenesis. Crnic, Ivana; Strittmatter, Karin; Cavallaro, Ugo; Kopfstein, Lucie; Jussila, Lotta; **Alitalo, Kari**; Christofori, Gerhard (Institute of Biochemistry and Genetics, Department of Clinical-Biological Sciences, University of Basel, Basel, Switz.). Cancer Research, 64(23), 8630-8638 (English) 2004. CODEN: CNREA8. ISSN: 0008-5472. Publisher: American Association for Cancer Research.

- AB Reduced expression of neural cell adhesion mol. (NCAM) has been implicated in the progression to tumor malignancy in cancer patients. Previously, we have shown that the loss of NCAM function causes the formation of lymph node metastasis in a transgenic mouse model of pancreatic β cell carcinogenesis (Rip1Tag2). Here we show that tumors of NCAM-deficient Rip1Tag2 transgenic mice exhibit up-regulated expression of the lymphangiogenic factors vascular endothelial growth factor (**VEGF**)-C and -D (17% in wild-type vs. 60% in NCAM-deficient Rip1Tag2 mice) and, with it, increased lymphangiogenesis (0% in wild-type vs. 19% in NCAM-deficient Rip1Tag2 mice). Repression of **VEGF-C** and -D function by adenoviral expression of a soluble form of their cognate receptor, VEGF receptor-3, results in reduced tumor lymphangiogenesis (56% vs. 28% in control vs. treated mice) and lymph node metastasis (36% vs. 8%

in control vs. treated mice). The results indicate that the loss of NCAM function causes lymph node metastasis via **VEGF-C**- and VEGF-D-mediated lymphangiogenesis. These results also establish Rip1Tag2;NCAM-deficient mice as a unique model for stochastic, endogenous tumor lymphangiogenesis and lymph node metastasis in immunocompetent mice.

L32 ANSWER 20 OF 121 CAPLUS COPYRIGHT 2006 ACS on STN

2004:885672 Document No. 141:392291 Molecular mechanisms of lymphatic development. **Kubo, Hajime** (Mol. Cancer Res. Unit., HMRO, Grad. Sch. Med., Kyoto Univ., Kyoto, 606-8501, Japan). Seikagaku, 76(9), 1210-1216 (Japanese) 2004. CODEN: SEIKAQ. ISSN: 0037-1017. Publisher: Nippon Seikagakkai.

AB A review on mols. common to vasculogenesis and lymphangiogenesis, e.g. **VEGF-C, D/VEGFR-3**, neuropilin-2, and angiopoietin-2, lymph duct-specific mols. LYVE-1 as a hyaluronan receptor and podoplanin, differentiation of lymphatic endothelial cells, genetic profiles of lymphatic endothelial cells, tumor-associated lymphangiogenesis and lymph node metastasis, possibility of preventing lymphangiogenesis and lymph node metastasis by administration of anti-**VEGFR-3** antibody, and treatment of mouse lymphedema model by **VEGF-C** gene therapy.

L32 ANSWER 21 OF 121 CAPLUS COPYRIGHT 2006 ACS on STN

2004:794639 Document No. 141:307616 Basic study of lymphangiogenesis and diseases. **Kubo, Hajime** (Grad. Sch. Med., Kyoto Univ., Japan). Ensho to Men'eki, 12(5), 608-615 (Japanese) 2004. CODEN: ENMEFA. ISSN: 0918-8371. Publisher: Sentan Igakusha.

AB A review on mol. mechanism of lymphangiogenesis and angiogenic factors, such as **VEGF-C/VEGFR-3** involved therein, differentiation of lymphatic endothelial cells, tumor metastasis to lymph node and anti-lymphangiogenesis therapy targeting **VEGF-C**, and lymphedema and lymphangiogenesis therapy.

L32 ANSWER 22 OF 121 MEDLINE on STN DUPLICATE 7

2004178418. PubMed ID: 15072591. Suppression of **VEGFR-3** signaling inhibits lymph node metastasis in gastric cancer. Shimizu Kenji; **Kubo Hajime**; Yamaguchi Koji; Kawashima Kazuhiko; Ueda Yoshihide; Matsuo Koichi; Awane Masaaki; Shimahara Yasuyuki; Takabayashi Arimichi; Yamaoka Yoshio; Satoh Seiji. (Department of Gastroenterological Surgery, Graduate School of Medicine, Kyoto University, Sakyo-ku, Kyoto 606-8507, Japan.) Cancer science, (2004 Apr) Vol. 95, No. 4, pp. 328-33. Journal code: 101168776. ISSN: 1347-9032. Pub. country: Japan. Language: English.

AB In gastric cancer, lymph node metastasis is one of the major prognostic factors and forms the basis for surgical removal of local lymph nodes. Recently, several studies have demonstrated that overexpression of lymphangiogenic growth factor **VEGF-C** or VEGF-D induces tumor lymphangiogenesis and promotes lymphatic metastasis in mouse tumor models. We examined whether these processes could be inhibited in naturally metastatic tumors by blocking of their cognate receptor **VEGFR-3** signaling pathway. Using a mouse orthotopic gastric cancer model which has a high frequency of lymph node metastasis, we estimated lymphatic vessels in gastric cancers by immunostaining for **VEGFR-3** and other specific lymphatic markers, LYVE-1 and prox-1. Then we systemically administered anti-**VEGFR-3** blocking antibodies. This treatment resulted in the inhibition of regional lymph node metastasis and reduction of lymphatic vessel density in the primary tumors. In addition, increased density of LYVE-1-positive lymphatic vessels of primary tumors was closely correlated with lymph node metastasis in human samples of gastric cancer. Antilymphangiogenesis by inhibiting **VEGFR-3** signaling could provide a potential strategy for the prevention of lymph node metastasis in gastric cancer.

L32 ANSWER 23 OF 121 MEDLINE on STN

DUPLICATE 8

2004296079. PubMed ID: 15197769. Immunodetection and quantification of vascular endothelial growth factor receptor-3 in human malignant tumor tissues. Bando Hiroko; Brokelmann Maren; Toi Masakazu; **Alitalo Kari**; Sleeman Jonathan P; Sipos Bence; Grone Hermann-Josef; Weich Herbert A. (Department of Gene Regulation and Differentiation, National Research Centre for Biotechnology (GBF), Braunschweig, Germany.) International journal of cancer. Journal international du cancer, (2004 Aug 20) Vol. 111, No. 2, pp. 184-91. Journal code: 0042124. ISSN: 0020-7136. Pub. country: United States. Language: English.

AB Vascular endothelial growth factor receptor-3 (**VEGFR-3**) and its ligands, vascular endothelial growth factor-C (**VEGF-C**) and -D (**VEGF-D**), are the major molecules involved in developmental and pathological lymphangiogenesis. Here we describe for the first time the development of a specific indirect enzyme-linked immunosorbent assay (ELISA) for the quantification of **VEGFR-3** in different human cell and tissue lysates. A combination of the goat polyclonal anti-**VEGFR-3** antibody and the mouse monoclonal anti-human **VEGFR-3** antibody was used. The assay was highly sensitive and reproducible with a detection range of 0.2-25 ng/ml. The assay was specific for **VEGFR-3**, with no cross-reactivity to VEGFR-1 or VEGFR-2. Complex formation with **VEGF-C** and VEGF-D had no effect on the sensitivity of the assay. The **VEGFR-3** concentration in the lysates of cultured human dermal microvascular endothelial cells was 14-fold higher than in the lysates from human umbilical vein endothelial cells. In human kidney, breast, colon, gastric and lung cancer tissues the protein levels of **VEGFR-3** were in the range of 0.6-16.7 ng/mg protein. Importantly, the level of **VEGFR-3** protein detected in the ELISA correlated significantly with the number of **VEGFR-3** positive vessels observed in histochemical sections, suggesting that the ELISA assay may be a reliable surrogate of measuring **VEGFR-3**-positive vessel density. The protein levels of **VEGFR-3** in 27 renal cell carcinoma samples had a significant correlation with the levels of **VEGF-C** ($p < 0.001$), or biological active, free VEGF-A ($p < 0.0001$), but not with VEGFR-1 or total VEGF-A. This assay provides a useful tool for the investigations of the expression levels of **VEGFR-3** in physiological and pathological processes, particular in cancer and in lymphangiogenesis-related disease. Copyright 2004 Wiley-Liss, Inc.

L32 ANSWER 24 OF 121 MEDLINE on STN DUPLICATE 9

2004091383. PubMed ID: 14980429. Quantification of vascular endothelial growth factor-C (**VEGF-C**) by a novel ELISA. Weich Herbert A; Bando Hiroko; Brokelmann Maren; Baumann Petra; Toi Masakazu; Barleon Bernhard; **Alitalo Kari**; Sipos Bence; Sleeman Jonathan. (Department of Gene Regulation and Differentiation National Research Centre for Biotechnology (GBF), Mascheroder Weg 1, 38124 Braunschweig, Germany.. weich@gbf.de) . Journal of immunological methods, (2004 Feb 15) Vol. 285, No. 2, pp. 145-55. Journal code: 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB Lymphangiogenesis plays an important role in several normal and pathological conditions such as wound healing, inflammation or metastasis formation in several malignancies. **VEGF-C** and VEGF-D are important and specific regulatory factors for lymphatic endothelial proliferation and lymphangiogenesis. In order to develop a highly sensitive and specific detection system for **VEGF-C**, we produced soluble binding proteins and antibodies for a microtiterplate-based assay. Here we describe a specific enzyme-linked immunosorbent assay (ELISA) for the measurement of human, rat and murine **VEGF-C**. The different antibodies developed against human and rat **VEGF-C** could be combined to detect processed and partially processed **VEGF-C** in a specific

way. The ELISA was able to detect human and rat **VEGF-C** with a minimum detection limit of 100 pg/ml. The assay did not show any cross-reactivity with the related protein VEGF-D. Furthermore, complex formation with its soluble receptors VEGFR-2 and **VEGFR-3** did not restricted the sensitivity of the assay. Using this assay, **VEGF-C** was measured in supernatants and lysates of different cell types and in tumour tissue samples of murine, rat and human origin. Cell lines secrete **VEGF-C** in very low amounts (<1 ng/ml) whereas **VEGF-C** transfected cells can secrete up to 50 ng/ml **VEGF-C** into the supernatant. In human tumour tissue samples **VEGF-C** was detected in some carcinomas in the low protein range. This ELISA will be a useful tool for investigations concerning the physiological function of **VEGF-C** in lymphangiogenesis under normal and pathophysiological conditions.

L32 ANSWER 25 OF 121 CAPLUS COPYRIGHT 2006 ACS on STN

2005:1179419 Document No. 144:184793 Plasmin activates **VEGF-C** and VEGF-D. McColl, Bradley K.; Baldwin, Megan E.; Roufail, Sally; Freeman, Craig; **Alitalo, Kari**; Stacker, Steven A.; Achen, Marc G. (Angiogenesis Laboratory, Ludwig Institute for Cancer Research, Royal Melbourne Hospital, Melbourne, Victoria, 3050, Australia). International Congress Series, 1262(Atherosclerosis XIII), 79-82 (English) 2004. CODEN: EXMDA4. ISSN: 0531-5131. Publisher: Elsevier B.V..

AB A review. Angiogenesis and lymphangiogenesis (growth of lymphatic vessels) are guided by members of the vascular endothelial growth factor (VEGF) family of secreted glycoproteins. In particular, **VEGF-C** and VEGF-D promote lymphangiogenesis as demonstrated in transgenic animal models and gene delivery studies. **VEGF-C** and VEGF-D are secreted as full-length forms that require proteolytic activation for high affinity binding to VEGF receptor-2 (VEGFR-2) and **VEGFR-3**, cell surface receptor tyrosine kinases localized on the endothelial cells of blood vessels and lymphatics. The proteases that activate these lymphangiogenic growth factors are key regulators of lymphatic development but were previously unknown. Here we report identification of the serine protease plasmin as an enzyme capable of activating both **VEGF-C** and VEGF-D.

L32 ANSWER 26 OF 121 MEDLINE on STN DUPLICATE 10

2004389867. PubMed ID: 15293565. Molecular mechanisms of lymphangiogenesis. Takahashi Meiko; Yoshimoto Takanobu; **Kubo Hajime**. (Molecular & Cancer Research Unit, HMRO, Graduate School of Medicine, Kyoto University, Kyoto, Japan.) International journal of hematology, (2004 Jul) Vol. 80, No. 1, pp. 29-34. Ref: 40. Journal code: 9111627. ISSN: 0925-5710. Pub. country: United States. Language: English.

AB Although the process of vascular development has been well documented, little is understood about lymphatic vasculature formation, despite its importance in normal and pathologic conditions. The dysfunction or abnormal growth of lymphatic vessels is associated with lymphedema and cancer metastasis. The recent discovery of lymphangiogenic growth factors vascular endothelial growth factor (**VEGF**)-**C** and VEGF-D and of their receptor, **VEGFR-3**, on lymphatic endothelial cells has started to provide an understanding of the molecular mechanisms of lymphangiogenesis. In addition, other genes that participate in the specification of lymphatic endothelial cells and the modulation of lymphatic vascular development have been identified. The capacity to induce or inhibit lymphangiogenesis by the manipulation of such molecules offers new opportunities to understand the function of the lymphatic system and to develop novel treatments for lymphatic disorders. This review describes the main players in lymphangiogenesis that have been identified so far and the attempts to shed some light on the mysteries surrounding this process.

L32 ANSWER 27 OF 121 MEDLINE on STN DUPLICATE 11
2004095074. PubMed ID: 14984763. Role of lymphangiogenic factors in tumor metastasis. He Yulong; Karpanen Terhi; **Alitalo Kari**. (Molecular/Cancer Biology Laboratory and Ludwig Institute for Cancer Research, Biomedicum Helsinki and Helsinki University Central Hospital, University of Helsinki, POB 63 (Haartmaninkatu 8), 00014 Helsinki, Finland.) *Biochimica et biophysica acta*, (2004 Mar 4) Vol. 1654, No. 1, pp. 3-12. Ref: 111. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.

AB Nearly four centuries after the discovery of lymphatic vessels, the molecular mechanisms underlying their development are beginning to be elucidated. Vascular endothelial growth factor C (**VEGF-C**) and VEGF-D, via signaling through **VEGFR-3**, appear to be essential for lymphatic vessel growth. Observations from clinicopathological studies have suggested that lymphatic vessels serve as the primary route for the metastatic spread of tumor cells to regional lymph nodes. Recent studies in animal models have provided convincing evidence that tumor lymphangiogenesis facilitates lymphatic metastasis. However, it is not clear how tumor-associated lymphangiogenesis is regulated, and little is known about how tumor cells escape from the primary tumor and gain entry into the lymphatics. This review examines some of these issues and provides a brief summary of the recent developments in this field of research.

L32 ANSWER 28 OF 121 CAPLUS COPYRIGHT 2006 ACS on STN
2003:282828 Document No. 138:298132 Modulators of VEGF or VEGFR binding to neuropilin-2, materials and methods for detecting said modulators, and therapeutic uses of the modulators.. **Alitalo, Kari**; Karkkainen, Marika; Karila, Kaisa (Ludwig Institute for Cancer Research, USA; Licentia Ltd.). PCT Int. Appl. WO 2003029814 A2 20030410, 181 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-EP11069 20021001. PRIORITY: US 2001-2001/PV326326 20011001.

AB The present invention relates to identifying modulators of **VEGF-C** binding to the nervous system transmembrane protein neuropilin-2 and materials and methods for detecting said modulators. A method of screening for modulators of binding between a neuropilin growth factor receptor and a **VEGF-C** polypeptide is claimed comprising steps of: (a) contacting a neuropilin composition with a **VEGF-C** composition, in the presence and in the absence of a putative modulator compound; (b) detecting binding between the neuropilin polypeptide and the **VEGF-C** polypeptide in the presence and absence of the putative modulator compound; and (c) identifying a modulator compound based on a decrease or increase in binding in the presence of the putative modulator compound as compared to binding in the absence of the putative modulator compound. The neuropilin receptor composition comprises a neuropilin receptor extracellular domain fragment bound to a solid support or a neuropilin receptor extracellular domain fragment fused to an Ig Fc fragment. The **VEGF-C** composition comprises a purified mammalian prepro-**VEGF-C** polypeptide or a fragment. A method of screening for modulators of binding between a neuropilin growth factor receptor and a **VEGFR-3** polypeptide is also claimed. The **VEGFR-3** composition used in the method comprises a receptor extracellular domain fragment bound to a solid support or a receptor extracellular domain fragment fused to an Ig Fc fragment. Addnl. claimed is a method for screening for selectivity of

a modulator of **VEGF-C**, VEGFR, or neuropilin biol. activity. A method of modulating growth, migration, or proliferation of cells, specifically neurons, in a mammalian organism by administering a composition comprising a neuropilin polypeptide or fragment, and a VEGF, a PlGF, a semaphorin, or a bispecific antibody specific for the neuropilin receptor and for a **VEGF-C** polypeptide or for a neuropilin receptor and a VEGFR is also claimed.

L32 ANSWER 29 OF 121 CAPLUS COPYRIGHT 2006 ACS on STN

2004:502659 Document No. 141:20142 Lymphatic endothelial cells materials and methods. **Alitalo, Kari**; Makinen, Taija (Ludwig Institute for Cancer Research, USA; Licentia, Ltd.). PCT Int. Appl. WO 2003006104 A2 20030123, 112 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US22164 20020712. PRIORITY: US 2001-2001/PV30488U 20010712; US 2001-2001/PV317610 20010906.

AB The present invention is directed to methods and compns. for isolating lymphatic endothelial cells from a mixed population of cells. More particularly, the inventors have found that certain antibodies that recognize the extracellular domain of **VEGFR-3** can be used to specifically isolated lymphatic endothelial cells substantially free of other contaminating non-lymphatic endothelial cells. Methods and compns. for producing such cells and using such cells are described.

L32 ANSWER 30 OF 121 CAPLUS COPYRIGHT 2006 ACS on STN

2003:887674 Document No. 139:359242 Production and uses of **VEGF-C** and other Flt4 receptor ligands. **Alitalo, Kari**; Joukov, Vladimir (Helsinki University Licensing Ltd. Oy, Finland; Ludwig Institute for Cancer Research). U.S. US 6645933 B1 20031111, 112 pp., Cont.-in-part of U.S. Ser. No. 601,132. (English). CODEN: USXXAM. APPLICATION: US 1996-671573 19960628. PRIORITY: US 1995-510133 19950801; US 1996-585895 19960112; US 1996-601132 19960214.

AB Provided are polypeptide ligands for the receptor tyrosine kinase, Flt4, including vascular endothelial growth factor (**VEGF-C**). Also provided are cDNAs and vectors encoding the ligands, pharmaceutical compns. and diagnostic reagents comprising the ligands, and methods of making and using the ligands.

L32 ANSWER 31 OF 121 MEDLINE on STN DUPLICATE 12

2003477600. PubMed ID: 12881528. Ligand-induced vascular endothelial growth factor receptor-3 (**VEGFR-3**) heterodimerization with VEGFR-2 in primary lymphatic endothelial cells regulates tyrosine phosphorylation sites. Dixelius Johan; Makinen Taija; Wirzenius Maria; Karkkainen Marika J; Wernstedt Christer; **Alitalo Kari**; Claesson-Welsh Lena. (Department of Genetics and Pathology, Uppsala University, Dag Hammarskjolds vag 20, S-751 85 Uppsala, Sweden.) The Journal of biological chemistry, (2003 Oct 17) Vol. 278, No. 42, pp. 40973-9. Electronic Publication: 2003-07-24. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Vascular endothelial growth factors (VEGFs) regulate the development and growth of the blood and lymphatic vascular systems. Of the three VEGF receptors (VEGFR), VEGFR-1 and -2 are expressed on blood vessels; VEGFR-2 is found also on lymphatic vessels. **VEGFR-3** is expressed mainly on lymphatic vessels but it is also up-regulated in tumor angiogenesis. Although **VEGFR-3** is essential for proper lymphatic development, its signal transduction mechanisms are still incompletely understood. Trans-phosphorylation of activated, dimerized

receptor tyrosine kinases is known to be critical for the regulation of kinase activity and for receptor interaction with signal transduction molecules. In this study, we have identified five tyrosyl phosphorylation sites in the **VEGFR-3** carboxyl-terminal tail. These sites were used both in **VEGFR-3** overexpressed in 293 cells and when the endogenous **VEGFR-3** was activated in lymphatic endothelial cells. Interestingly, **VEGF-C** stimulation of lymphatic endothelial cells also induced the formation of **VEGFR-3/VEGFR-2** heterodimers, in which **VEGFR-3** was phosphorylated only at three of the five sites while the two most carboxyl-terminal tyrosine residues appeared not to be accessible for the **VEGFR-2** kinase. Our data suggest that the carboxyl-terminal tail of **VEGFR-3** provides important regulatory tyrosine phosphorylation sites with potential signal transduction capacity and that these sites are differentially used in ligand-induced homo- and heterodimeric receptor complexes.

L32 ANSWER 32 OF 121 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

2003:1072948 The Genuine Article (R) Number: 750AA. Intrinsic versus micro environmental regulation of lymphatic endothelial cell phenotype and function. Veikkola T; Lohela M; Ikenberg K; Makinen T; Korff T; Saaristo A; Petrova T; Jeltsch M; Augustin H G; **Alitalo K (Reprint)**. Univ Helsinki, Biomedicum Helsinki, Mol Canc Biol Lab, POB 63, Haartmaninkatu 8, FIN-00014 Helsinki, Finland (Reprint); Univ Helsinki, Biomedicum Helsinki, Mol Canc Biol Lab, FIN-00014 Helsinki, Finland; Univ Helsinki, Biomedicum Helsinki, Ludwig Inst Canc Res, Haartman Inst, FIN-00014 Helsinki, Finland; Univ Helsinki, Cent Hosp, FIN-00014 Helsinki, Finland; Tumor Biol Ctr, Dept Vasc Biol & Angiogenesis Res, D-79106 Freiburg, Germany. FASEB JOURNAL (NOV 2003) Vol. 17, No. 14, pp. 2006-2013. ISSN: 0892-6638. Publisher: FEDERATION AMER SOC EXP BIOL, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3998 USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Vascular endothelial cells are characterized by a high degree of functional and phenotypic plasticity, which is controlled both by their pericellular microenvironment and their intracellular gene expression programs. To gain further insight into the mechanisms regulating the endothelial cell phenotype, we have compared the responses of lymphatic endothelial cells (LECs) and blood vascular endothelial cells (BECs) to vascular endothelial growth factors (VEGFs). **VEGFR-3**-specific signals are sufficient for LEC but not BEC proliferation, as shown by the ability of the specific ligand VEGF-C156S to stimulate cell cycle entry only in LECs. On the other hand, we found that **VEGFR-3** stimulation did not induce LEC cell shape changes typical of **VEGFR-2**-stimulated LECs, indicating receptor-specific differences in the cytoskeletal responses. Genes induced via **VEGFR-2** also differed between BECs and LECs: angiopoietin-2 (Ang-2) was induced via **VEGFR-2** in BECs and LECs, but the smooth muscle cell (SMC) chemoattractant BMP-2 was induced only in BECs. Both BECs and LECs were able to promote SMC chemotaxis, but contact with SMCs led to down-regulation of **VEGFR-3** expression in BECs in a 3-dimensional coculture system. This was consistent with the finding that **VEGFR-3** is down-regulated in vivo at sites of endothelial cell-pericyte/smooth muscle cell contacts. Collectively, these data show intrinsic cell-specific differences of BEC and LEC responses to VEGFs and identify a pericellular regulatory mechanism for **VEGFR-3** down-regulation in endothelial cells.

L32 ANSWER 33 OF 121 CAPLUS COPYRIGHT 2006 ACS on STN

2003:759487 Document No. 140:88061 Angiogenic responses of vascular endothelial growth factors in periadventitial tissue. Bhardwaj, Shalini; Roy, Himadri; Gruchala, Marcin; Viita, Helena; Kholova, Ivana; Kokina, Ilze; Achen, Marc G.; Stacker, Steven A.; Hedman, Marja; **Alitalo**,

Kari; Ylae-Herttuala, Seppo (A.I. Virtanen Institute for Molecular Sciences, Department of Biotechnology and Molecular Medicine, University of Kuopio, Kuopio, 70211, Finland). Human Gene Therapy, 14(15), 1451-1462 (English) 2003. CODEN: HGTHE3. ISSN: 1043-0342. Publisher: Mary Ann Liebert, Inc..

AB Recent discovery of new members of the vascular endothelial growth factor (VEGF) family has generated much interest as to which members may be best suited for therapeutic angiogenesis in various tissues. In this study we evaluated angiogenic responses of the different members of the VEGF family in vivo using adenoviral gene transfer. Adenoviruses (1+109 plaque-forming units [pfu]) encoding for VEGF-A, VEGF-B, **VEGF-C**, VEGF-D, **VEGF-C.DELTA.NAC** and VEGF-DANAC (Δ NAC are proteolytically cleaved forms) were transferred locally to the periadventitial space of the rabbit carotid arteries using a collar technique that allows efficient local transfection of the periadventitial tissue. Expression of the transfected VEGFs was confirmed by immunohistochem. and reverse transcription-polymerase chain reaction (RT-PCR). Seven days after the gene transfer maximum neovessel formation was observed in VEGF-A-, VEGF-D-, and VEGF-DANAC-transfected arteries. **VEGF-C** Δ NAC also showed angiogenic activity whereas VEGF-B was not effective in inducing angiogenesis. Pericytes were detected around the neovessels, which also frequently showed the presence of intraluminal erythrocytes. Infiltration of inflammatory cells in response to VEGF-D and VEGF-DANAC was less prominent than that caused by other VEGFs. In line with the absence of lymphatics in the normal carotid arteries no significant evidence of lymphatic vessel formation was seen in response to any of the studied VEGFs in the periadventitial space. The results help to define possibilities for local angiogenic therapy around blood vessels and support the concept that angiogenic effects may be tissue-specific and depend both on the growth factor ligands and the target tissues. It is concluded that VEGF-A, VEGF-D, and VEGF-DANAC are the best candidates for therapeutic angiogenesis when delivered around large arteries.

L32 ANSWER 34 OF 121 MEDLINE on STN DUPLICATE 13
2003081301. PubMed ID: 12393458. Modulation of VEGFR-2-mediated endothelial-cell activity by **VEGF-C/VEGFR-3**. Matsumura Kazuyoshi; Hirashima Masanori; Ogawa Minetaro; **Kubo Hajime**; Hisatsune Hiroshi; Kondo Nobuyuki; Nishikawa Satomi; Chiba Tsutomu; Nishikawa Shin-Ichi. (Division of Gastroenterology and Hepatology, Department of Internal Medicine, Graduate School of Medicine, Kyoto University, Japan.. kazuy@kuhp.hyoto-u.ac.jp) . Blood, (2003 Feb 15) Vol. 101, No. 4, pp. 1367-74. Electronic Publication: 2002-10-10. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB Vascular endothelial growth factor (VEGF) receptor 3 (**VEGFR-3**), a receptor for **VEGF-C**, was shown to be essential for angiogenesis as well as for lymphangiogenesis. Targeted disruption of the **VEGFR-3** gene in mice and our previous study using an antagonistic monoclonal antibody (MoAb) for **VEGFR-3** suggested that **VEGF-C/VEGFR-3** signals might be involved in the maintenance of vascular integrity. In this study we used an in vitro embryonic stem (ES) cell culture system to maintain the **VEGFR-3**(+) endothelial cell (EC) and investigated the role of **VEGFR-3** signals at the cellular level. In this system packed clusters of ECs were formed. Whereas addition of exogenous VEGF-A induced EC dispersion, **VEGF-C**, which can also stimulate VEGFR-2, promoted EC growth without disturbing the EC clusters. Moreover, addition of AFL4, an antagonistic MoAb for **VEGFR-3**, resulted in EC dispersion. Cytological analysis showed that VEGF-A- and AFL4-treated ECs were indistinguishable in many aspects but were distinct from the

cytological profile induced by antagonistic MoAb for VE-cadherin (VECD-1). As AFL4- induced EC dispersion requires VEGF-A stimulation, it is likely that **VEGFR-3** signals negatively modulate VEGFR-2. This result provides new insights into the involvement of **VEGFR-3** signals in the maintenance of vascular integrity through modulation of VEGFR-2 signals. Moreover, our findings suggest that the mechanisms underlying AFL4-induced EC dispersion are distinct from those underlying VECD-1-induced dispersion for maintenance of EC integrity.

L32 ANSWER 35 OF 121 CAPLUS COPYRIGHT 2006 ACS on STN

2003:801321 Document No. 141:82808 Plasmin activates the lymphangiogenic growth factors **VEGF-C** and VEGF-D. [Erratum to document cited in CA140:013416]. McColl, Bradley K.; Baldwin, Megan E.; Roufail, Saly; Freeman, Craig; Moritz, Robert L.; Simpson, Richard J.; **Alitalo, Kari**; Stacker, Steven A.; Achen, Marc G. (Ludwig Institute for Cancer Research, Royal Melbourne Hospital, Melbourne, 3050, Australia). Journal of Experimental Medicine, 198(7), 1127 (English) 2003. CODEN: JEMEAU. ISSN: 0022-1007. Publisher: Rockefeller University Press.

AB In the Results section, subheading Assay for VEGF-D Processing, "kD" was used instead of the unit "D", implying that the value being quoted was 1000-fold greater than in reality. The corrected paragraph is given.

L32 ANSWER 36 OF 121 MEDLINE on STN DUPLICATE 14

2003251849. PubMed ID: 12714562. VEGF-D is the strongest angiogenic and lymphangiogenic effector among VEGFs delivered into skeletal muscle via adenoviruses. Rissanen Tuomas T; Markkanen Johanna E; Gruchala Marcin; Heikura Tommi; Puranen Antti; Kettunen Mikko I; Kholova Ivana; Kauppinen Risto A; Achen Marc G; Stacker Steven A; **Alitalo Kari**; Yla-Herttuala Seppo. (Department of Biotechnology and Molecular Medicine, A.I. Virtanen Institute, University of Kuopio, PO Box 1627, FIN-70211 Kuopio, Finland.) Circulation research, (2003 May 30) Vol. 92, No. 10, pp. 1098-106. Electronic Publication: 2003-04-24. Journal code: 0047103. E-ISSN: 1524-4571. Pub. country: United States. Language: English.

AB Optimal angiogenic and lymphangiogenic gene therapy requires knowledge of the best growth factors for each purpose. We studied the therapeutic potential of human vascular endothelial growth factor (VEGF) family members VEGF-A, VEGF-B, **VEGF-C**, and VEGF-D as well as a **VEGFR-3**-specific mutant (VEGF-C156S) using adenoviral gene transfer in rabbit hindlimb skeletal muscle. The significance of proteolytic processing of VEGF-D was explored using adenoviruses encoding either full-length or mature (DeltaNDeltaC) VEGF-D. Adenoviruses expressing potent VEGFR-2 ligands, VEGF-A and VEGF-DDeltaNDeltaC, induced the strongest angiogenesis and vascular permeability effects as assessed by capillary vessel and perfusion measurements, modified Miles assay, and MRI. The most significant feature of angiogenesis induced by both VEGF-A and VEGF-DDeltaNDeltaC was a remarkable enlargement of microvessels with efficient recruitment of pericytes suggesting formation of arterioles or venules. VEGF-A also moderately increased capillary density and created glomeruloid bodies, clusters of tortuous vessels, whereas VEGF-DDeltaNDeltaC-induced angiogenesis was more diffuse. Vascular smooth muscle cell proliferation occurred in regions with increased plasma protein extravasation, indicating that arteriogenesis may be promoted by VEGF-A and VEGF-DDeltaNDeltaC. Full-length **VEGF-C** and VEGF-D induced predominantly and the selective **VEGFR-3** ligand VEGF-C156S exclusively lymphangiogenesis. Unlike angiogenesis, lymphangiogenesis was not dependent on nitric oxide. The VEGFR-1 ligand VEGF-B did not promote either angiogenesis or lymphangiogenesis. Finally, we found a positive correlation between capillary size and vascular permeability. This study compares, for the first time, angiogenesis and lymphangiogenesis induced by gene transfer of different human VEGFs, and shows that VEGF-D is the most potent member when delivered via an

adenoviral vector into skeletal muscle.

- L32 ANSWER 37 OF 121 MEDLINE on STN DUPLICATE 15
2003434367. PubMed ID: 12963694. Plasmin activates the lymphangiogenic growth factors **VEGF-C** and VEGF-D. McColl Bradley K; Baldwin Megan E; Roufail Sally; Freeman Craig; Moritz Robert L; Simpson Richard J; **Alitalo Kari**; Stacker Steven A; Achen Marc G. (Ludwig Institute for Cancer Research, Royal Melbourne Hospital, Melbourne, Victoria 3050, Australia.. Marc.achen@ludwig.edu.au) . The Journal of experimental medicine, (2003 Sep 15) Vol. 198, No. 6, pp. 863-8. Electronic Publication: 2003-09-08. Journal code: 2985109R. ISSN: 0022-1007. Pub. country: United States. Language: English.
- AB Vascular endothelial growth factor (**VEGF**) **C** and VEGF-D stimulate lymphangiogenesis and angiogenesis in tissues and tumors by activating the endothelial cell surface receptor tyrosine kinases VEGF receptor (VEGFR) 2 and **VEGFR-3**. These growth factors are secreted as full-length inactive forms consisting of NH2- and COOH-terminal propeptides and a central VEGF homology domain (VHD) containing receptor binding sites. Proteolytic cleavage removes the propeptides to generate mature forms, consisting of dimers of the VEGF homology domain, that bind receptors with much greater affinity than the full-length forms. Therefore, proteolytic processing activates **VEGF-C** and VEGF-D, although the proteases involved were unknown. Here, we report that the serine protease plasmin cleaved both propeptides from the VEGF homology domain of human VEGF-D and thereby generated a mature form exhibiting greatly enhanced binding and cross-linking of VEGFR-2 and **VEGFR-3** in comparison to full-length material. Plasmin also activated **VEGF-C**. As lymphangiogenic growth factors promote the metastatic spread of cancer via the lymphatics, the proteolytic activation of these molecules represents a potential target for antimetastatic agents. Identification of an enzyme that activates the lymphangiogenic growth factors will facilitate development of inhibitors of metastasis.
- L32 ANSWER 38 OF 121 MEDLINE on STN DUPLICATE 16
2003133070. PubMed ID: 12618526. **VEGF-C** gene therapy augments postnatal lymphangiogenesis and ameliorates secondary lymphedema. Yoon Young-Sup; Murayama Toshinori; Gravereaux Edwin; Tkebuchava Tengiz; Silver Marcy; Curry Cynthia; Wecker Andrea; Kirchmair Rudolf; Hu Chun Song; Kearney Marianne; Ashare Alan; Jackson David G; **Kubo Hajime**; Isner Jeffrey M; Losordo Douglas W. (Department of Vascular Medicine, St. Elizabeth's Medical Center, Tufts University School of Medicine, Boston, Massachusetts, USA.) The Journal of clinical investigation, (2003 Mar) Vol. 111, No. 5, pp. 717-25. Journal code: 7802877. ISSN: 0021-9738. Pub. country: United States. Language: English.
- AB Although lymphedema is a common clinical condition, treatment for this disabling condition remains limited and largely ineffective. Recently, it has been reported that overexpression of **VEGF-C** correlates with increased lymphatic vessel growth (lymphangiogenesis). However, the effect of **VEGF-C**-induced lymphangiogenesis on lymphedema has yet to be demonstrated. Here we investigated the impact of local transfer of naked plasmid DNA encoding human **VEGF-C** (phVEGF-C) on two animal models of lymphedema: one in the rabbit ear and the other in the mouse tail. In a rabbit model, following local phVEGF-C gene transfer, **VEGFR-3** expression was significantly increased. This gene transfer led to a decrease in thickness and volume of lymphedema, improvement of lymphatic function demonstrated by serial lymphoscintigraphy, and finally, attenuation of the fibrofatty changes of the skin, the final consequences of lymphedema. The favorable effect of phVEGF-C on lymphedema was reconfirmed in a mouse tail model. Immunohistochemical analysis using lymphatic-specific markers: **VEGFR-3**, lymphatic endothelial hyaluronan receptor-1, together with the proliferation marker

Ki-67 Ab revealed that phVEGF-C transfection potently induced new lymphatic vessel growth. This study, we believe for the first time, documents that gene transfer of phVEGF-C resolves lymphedema through direct augmentation of lymphangiogenesis. This novel therapeutic strategy may merit clinical investigation in patients with lymphedema.

L32 ANSWER 39 OF 121 CAPLUS COPYRIGHT 2006 ACS on STN

2003:1005440 Document No. 140:87753 **VEGF-C/VEGFR**

-3 and lymphangiogenesis. **Kubo, Hajime** (Grad. Sch. Med., Kyoto Univ., Japan). *Kekkan Igaku*, 4(6), 623-633 (Japanese) 2003. CODEN: KIEGA2. ISSN: 1345-9031. Publisher: Medikaru Rebyusha.

AB A review on mol. mechanism of lymphangiogenesis and factors, such as **VEGF-C/D**, **VEGFR-3**, angiopoietin/Tie, and ephrin B, etc., involved therein, genes involved in development and differentiation of the lymph vessel, tumor lymph node metastasis and anti-lymphangiogenesis therapy, and **VEGF-C** gene therapy inducing lymphangiogenesis in lymphedema.

L32 ANSWER 40 OF 121 MEDLINE on STN

DUPLICATE 17

2003:188024. PubMed ID: 12706123. Vascular endothelial growth factor (VEGF) receptor-2 signaling mediates **VEGF-C**(deltaNdeltaC)- and VEGF-A-induced angiogenesis in vitro. Tille Jean-Christophe; Wang Xueyan; Lipson Kenneth E; McMahon Gerald; Ferrara Napoleone; Zhu Zhenping; Hicklin Daniel J; Sleeman Jonathan P; Eriksson Ulf; **Alitalo Kari**; Pepper Michael S. (Department of Cell Biology and Morphology, University Medical Center, Geneva, Switzerland.) *Experimental cell research*, (2003 May 1) Vol. 285, No. 2, pp. 286-98. Journal code: 0373226. ISSN: 0014-4827. Pub. country: United States. Language: English.

AB Angiogenesis and lymphangiogenesis are regulated by members of the vascular endothelial growth factor (VEGF) family of cytokines, which mediate their effects via tyrosine kinase VEGF receptors -1, -2, and -3. We have used wild-type and mutant forms of VEGFs -A, -B, and -C, a pan-VEGFR tyrosine kinase inhibitor (SU5416) as well as neutralizing anti-VEGFR-2 antibodies, to determine which VEGF receptor(s) are required for bovine endothelial cell invasion and tube formation in vitro. This was compared to the ability of these cytokines to induce expression of members of the plasminogen activator (PA)-plasmin system. We found that cytokines which bind VEGFR-2 (human VEGF-A, human VFM23A, human **VEGF-C**(deltaNdeltaC), and rat **VEGF-C** (152)) induced invasion, tube formation, urokinase-type-PA, tissue-type-PA, and PA inhibitor-1, invasion and tube formation as well as signaling via the MAP kinase pathway were efficiently blocked by SU5416 and anti-VEGFR-2 antibodies. In contrast, cytokines and mutants which exclusively bind VEGFR-1 (human VFM17 and human VEGF-B) had no effect on invasion and tube formation or on the regulation of gene expression. We were unable to identify cytokines which selectively stimulate bovine **VEGFR-3** in our system. Taken together, these findings point to the central role of VEGFR-2 in the angiogenic signaling pathways induced by **VEGF-C**(deltaNdeltaC) and VEGF-A.

L32 ANSWER 41 OF 121 MEDLINE on STN

DUPLICATE 18

2003:356155. PubMed ID: 12888864. Lymphangiogenic growth factors, receptors and therapies. Lohela Marja; Saaristo Anne; Veikkola Tanja; **Alitalo Kari**. (Molecular/Cancer Biology Laboratory and Ludwig Institute for Cancer Research, Haartman Institute and Helsinki University Central Hospital, Biomedicum Helsinki, University of Helsinki, 00014 Helsinki, Finland.) *Thrombosis and haemostasis*, (2003 Aug) Vol. 90, No. 2, pp. 167-84. Ref: 226. Journal code: 7608063. ISSN: 0340-6245. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB The lymphatic vasculature is essential for the maintenance of normal fluid balance and for the immune responses, but it is also involved in a variety of diseases. Hypoplasia or dysfunction of the lymphatic vessels can lead to lymphedema, whereas hyperplasia or abnormal growth of these vessels are

associated with lymphangiomas and lymphangiosarcomas. Lymphatic vessels are also involved in lymph node and systemic metastasis of cancer cells. Recent novel findings on the molecular mechanisms involved in lymphatic vessel development and regulation allow the modulation of the lymphangiogenic process and specific targeting of the lymphatic endothelium. Recent results show that the homeodomain transcription factor Prox-1 is an important lymphatic endothelial cell (LEC) fate-determining factor which can induce LEC-specific gene transcription even in blood vascular endothelial cells (BECs). This suggests that the distinct phenotypes of cells in the adult vascular endothelium are plastic and sensitive to transcriptional reprogramming, which might be useful for future therapeutic applications involving endothelial cells. Vascular endothelial growth factor-C (**VEGF-C**) and VEGF-D are peptide growth factors capable of inducing the growth of new lymphatic vessels in vivo in a process called lymphangiogenesis. They belong to the larger family which also includes VEGF, placenta growth factor (PlGF) and VEGF-B, **VEGF-C** and VEGF-D are ligands for the endothelial cell specific tyrosine kinase receptors VEGFR-2 and **VEGFR-3**. In adult human as well as mouse tissues **VEGFR-3** is expressed predominantly in lymphatic endothelial cells which line the inner surface of lymphatic vessels. While VEGFR-2 is thought to be the main mediator of angiogenesis, **VEGFR-3** signaling is crucial for the development of the lymphatic vessels. Heterozygous inactivation of the **VEGFR-3** tyrosine kinase leads to primary lymphedema due to defective lymphatic drainage in the limbs. Other factors that seem to be involved in lymphangiogenesis include the Tie/angiopoietin system, neuropilin-2 and integrin alpha 9. **VEGF-C** induces lymphatic vessel growth, but high levels of **VEGF-C** also resulted in blood vessel leakiness and growth. The **VEGFR-3**-specific mutant form of **VEGF-C** called VEGF-C156S lacks blood vascular side effects but is sufficient for therapeutic lymphangiogenesis in a mouse model of lymphedema. As VEGF-C156S is a specific lymphatic endothelial growth factor in the skin, it provides an attractive molecule for pro-lymphangiogenic therapy.

L32 ANSWER 42 OF 121 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

2003:992107 The Genuine Article (R) Number: 742WV. Genesis and pathogenesis of lymphatic vessels. Jeltsch M; Tammela T; **Alitalo K (Reprint)**; Wilting J. Ludwig Inst Canc Res, Mol & Canc Biol Lab, Haartmaninkatu 8, Postbox 63, Helsinki 00014, Finland (Reprint); Ludwig Inst Canc Res, Mol & Canc Biol Lab, Helsinki 00014, Finland; Univ Helsinki, Cent Hosp, Biomedicum Helsinki, Helsinki 00014, Finland; Childrens Hosp, D-37075 Gottingen, Germany. CELL AND TISSUE RESEARCH (OCT 2003) Vol. 314, No. 1, pp. 69-84. ISSN: 0302-766X. Publisher: SPRINGER-VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The lymphatic system is generally regarded as supplementary to the blood vascular system, in that it transports interstitial fluid, macromolecules, and immune cells back into the blood. However, in insects, the open hemolymphatic (or lymphohematic) system ensures the circulation of immune cells and interstitial fluid through the body. The *Drosophila* homolog of the mammalian vascular endothelial growth factor receptor (VEGFR) gene family is expressed in hemocytes, suggesting a close relationship to the endothelium that develops later in phylogeny. Lymph hearts are typical organs for the propulsion of lymph in lower vertebrates and are still transiently present in birds. The lymphatic endothelial marker **VEGFR-3** is transiently expressed in embryonic blood vessels and is crucial for their development. We therefore regard the question of whether the blood vascular system or the lymphatic system is primary or secondary as open. Future molecular comparisons should be performed without any bias based on the current prevalence of the blood

vascular system over the lymphatic system. Here, we give an overview of the structure, function, and development of the lymphatics, with special emphasis on the recently discovered lymphangiogenic growth factors.

L32 ANSWER 43 OF 121 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

2004:100284 Document No.: PREV200400097584. Lymphangiogenic growth factors and tumor metastasis. **Alitalo, K.** [Reprint Author]. Molecular/Cancer Biology, University of Helsinki Haartman Institute, Helsinki, Finland. EJC Supplements, (September 2003) Vol. 1, No. 5, pp. S25. print. Meeting Info.: 12th ECCO (European Cancer Conference). Copenhagen, Denmark. September 21-25, 2003. Federation of European Cancer Societies. ISSN: 1359-6349 (ISSN print). Language: English.

L32 ANSWER 44 OF 121 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN DUPLICATE 19

2002:389236 Document No.: PREV200200389236. Antibodies reactive with **VEGF-C**, a ligand for the Flt4 receptor tyrosine kinase (**VEGFR-3**). **Alitalo, Kari** [Inventor, Reprint author]; Joukov, Vladimir [Inventor]. Espoo, Finland. ASSIGNEE: Helsinki University Licensing, Ltd., Helsinki, Finland; Ludwig Institute for Cancer Research. Patent Info.: US 6403088 20020611. Official Gazette of the United States Patent and Trademark Office Patents, (June 11, 2002) Vol. 1259, No. 2. <http://www.uspto.gov/web/menu/patdata.html>. e-file. CODEN: OGUPE7. ISSN: 0098-1133. Language: English.

AB The invention provides antibodies that are reactive with **VEGF-C**, a polypeptide ligand for Flt4 receptor tyrosine kinase (**VEGFR-3**).

L32 ANSWER 45 OF 121 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN DUPLICATE 20

2002:279710 Document No.: PREV200200279710. Vascular endothelial growth factor C (**VEGF-C**) protein and gene, mutants thereof, and uses thereof. **Alitalo, Kari** [Inventor, Reprint author]; Joukov, Vladimir [Inventor]. Helsinki, Finland. ASSIGNEE: Licentia Ltd, Helsinki, Finland; Ludwig Institute for Cancer Research. Patent Info.: US 6361946 20020326. Official Gazette of the United States Patent and Trademark Office Patents, (Mar. 26, 2002) Vol. 1256, No. 4. <http://www.uspto.gov/web/menu/patdata.html>. e-file. CODEN: OGUPE7. ISSN: 0098-1133. Language: English.

AB Provided are purified and isolated **VEGF-C** polypeptides capable of binding to at least one of KDR receptor tyrosine kinase (**VEGFR-2**) and Flt4 receptor tyrosine kinase (**VEGFR-3**); analogs of such peptides that have **VEGF-C**-like or **VEGF**-like biological activities or that are **VEGF** or **VEGF-C** inhibitors; polynucleotides encoding the polypeptides; vectors and host cells that embody the polynucleotides; pharmaceutical compositions and diagnostic reagents comprising the polypeptides; and methods of making and using the polypeptides.

L32 ANSWER 46 OF 121 CAPLUS COPYRIGHT 2006 ACS on STN

2002:594892 Document No. 137:150622 Cloning, tissue expression and therapeutic use of Flt4 (**VEGFR-3**) polypeptides, their polynucleotides, and antibodies in the diagnosis and treatment of cancer. **Alitalo, Kari; Aprelikova, Olga; Pajusola, Katri; Armstrong, Elina; Korhonen, Jaana; Kaipainen, Arja** (Ludwig Institute for Cancer Research, USA). PCT Int. Appl. WO 2002060950 A2 20020808, 173 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI,

FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR.
(English). CODEN: PIXXD2. APPLICATION: WO 2002-US1784 20020122.
PRIORITY: US 1994-340011 19941114; US 1994-169079 19941114; US 1997-901710
19970728; US 2001-2001/765534 20010119.

AB The present invention provide purified Flt4 receptor tyrosine kinase polypeptides and fragments thereof, polynucleotides encoding such polypeptides, antibodies that specifically bind such polypeptides, and uses thereof in the treatment and diagnosis of disease, specifically cancer.

L32 ANSWER 47 OF 121 CAPLUS COPYRIGHT 2006 ACS on STN
2002:555522 Document No. 137:119669 **VEGFR-3** inhibitor materials and methods. **Alitalo, Kari; Koivunen, Erkki; Kubo, Hajime** (Ludwig Institute for Cancer Research, USA; Licentia Ltd.). PCT Int. Appl. WO 2002057299 A2 20020725, 149 pp.
DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR.
(English). CODEN: PIXXD2. APPLICATION: WO 2002-IB99 20020116. PRIORITY: US 2001-2001/PV262476 20010117.

AB The present invention relates to the diagnostics, evaluation, and therapeutic intervention of disorders mediated by the activity of cell surface receptor **VEGFR-3**, which activity often is stimulated by **VEGFR-3** ligands **VEGF-C** and **VEGF-D**. More particularly, the present invention identifies novel methods and compns. for the inhibition of **VEGF-C/D** binding to **VEGFR-3**. The compns. of the present invention will be useful the inhibition of angiogenesis and lymphangiogenesis. Many uses of such compds., for screening samples, imaging, diagnosis, and therapy, are also provided. For example, in one embodiment, the invention provides an isolated peptide comprising the formula: X1X2X3X4X5X6X7X8, wherein X1 through X8 are amino acid residues.

L32 ANSWER 48 OF 121 MEDLINE on STN DUPLICATE 21
2002349623. PubMed ID: 12070340. Blockade of vascular endothelial growth factor receptor-3 signaling inhibits fibroblast growth factor-2-induced lymphangiogenesis in mouse cornea. **Kubo Hajime; Cao Renhai; Brakenhielm Ebba; Makinen Taija; Cao Yihai; Alitalo Kari.** (Molecular/Cancer Biology Laboratory and Ludwig Institute for Cancer Research, and Helsinki University Central Hospital, Biomedicum Helsinki, University of Helsinki, POB 63, Haartmaninkatu 8, 00014, Helsinki, Finland.) Proceedings of the National Academy of Sciences of the United States of America, (2002 Jun 25) Vol. 99, No. 13, pp. 8868-73. Electronic Publication: 2002-06-17. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Vascular endothelial growth factor receptor-3 (**VEGFR-3**) is a major mediator of lymphangiogenesis. Recently, **VEGFR-3** ligands, **VEGF-C**, and **VEGF-D** were reported to promote tumor lymphangiogenesis and lymphatic metastasis, and these processes were inhibited by blocking of the **VEGFR-3**-signaling pathway. Here, we have adapted the mouse corneal angiogenesis assay to study potential lymphangiogenic factors and inhibitors. Immunohistochemical analysis with lymphatic endothelial markers showed that **VEGF-C** induces lymphatic as well as blood vessel growth in the cornea. By contrast, VEGF induced angiogenesis but not lymphangiogenesis. Fibroblast growth factor-2 (FGF-2) stimulated both lymphangiogenesis and angiogenesis. FGF-2 up-regulated **VEGF-C** expression in vascular endothelial and perivascular cells. Furthermore, administration of blocking anti-**VEGFR-3**

antibodies inhibited the FGF-2-induced lymphangiogenesis. These findings show that **VEGFR-3** can mediate lymphangiogenesis induced by other growth factors. Because increased expression of FGF-2 and **VEGF-C** has been associated with lymphatic metastasis, our results provide a potential strategy for the inhibition of lymphatic metastasis in cancer therapy.

L32 ANSWER 49 OF 121 MEDLINE on STN DUPLICATE 22
2002145239. PubMed ID: 11877295. Vascular endothelial growth factor (**VEGF**)-C signaling through FLT-4 (**VEGFR-3**) mediates leukemic cell proliferation, survival, and resistance to chemotherapy. Dias Sergio; Choy Margaret; **Alitalo Kari**; Rafii Shahin. (Division of Hematology/Oncology, Weill Medical College of Cornell University, 1300 York Ave, New York, NY 10021, USA.) Blood, (2002 Mar 15) Vol. 99, No. 6, pp. 2179-84. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB Similar to solid tumors, growth of leukemias may also be angiogenesis dependent. Furthermore, tyrosine kinase receptors specific to endothelial cells are expressed on certain subsets of leukemias. We have previously demonstrated the existence of a VEGF/VEGFR-2 autocrine loop on leukemic cells that supports their growth and migration. Here, we demonstrate that in response to leukemia-derived proangiogenic and proinflammatory cytokines such as basic fibroblast growth factor and IL-1, endothelial cells release increasing amounts of another vascular endothelial growth factor (VEGF) family member, **VEGF-C**. In turn, interaction of **VEGF-C** with its receptor **VEGFR-3** (FLT-4) promotes leukemia survival and proliferation. We demonstrate in 2 cell lines and 5 FLT-4(+) leukemias that **VEGF-C** and a mutant form of the molecule that lacks the KDR-binding motif induce receptor phosphorylation, leukemia proliferation, and increased survival, as determined by increased Bcl-2/Bax ratios. Moreover, **VEGF-C** protected leukemic cells from the apoptotic effects of 3 chemotherapeutic agents. Because most leukemic cells release proangiogenic as well as proinflammatory cytokines, our data suggest that the generation of a novel paracrine angiogenic loop involving **VEGF-C** and FLT-4 may promote the survival of a subset of leukemias and protect them from chemotherapy-induced apoptosis. These results identify the **VEGF-C**/FLT-4 pathway as a novel therapeutic target for the treatment of subsets of acute leukemia.

L32 ANSWER 50 OF 121 MEDLINE on STN DUPLICATE 23
2002707683. PubMed ID: 12397087. Therapeutic lymphangiogenesis with human recombinant **VEGF-C**. Szuba Andrzej; Skobe Mihaela; Karkkainen Marika J; Shin William S; Beynet David P; Rockson Ned B; Dakhil Noma; Spilman Stan; Goris Michael L; Strauss H William; Quertermous Thomas; **Alitalo Kari**; Rockson Stanley G. (Division of Cardiovascular Medicine, Falk Cardiovascular Research Center, Stanford University School of Medicine, Stanford, California 94305, USA.) The FASEB journal : official publication of the Federation of American Societies for Experimental Biology, (2002 Dec) Vol. 16, No. 14, pp. 1985-7. Electronic Publication: 2002-10-18. Journal code: 8804484. E-ISSN: 1530-6860. Pub. country: United States. Language: English.

AB Chronic regional impairments of the lymphatic circulation often lead to striking architectural abnormalities in the lymphedematous tissues. Lymphedema is a common, disabling disease that currently lacks a cure. Vascular endothelial growth factors C and D mediate lymphangiogenesis through the **VEGFR-3** receptor on lymphatic endothelia. The purpose of this study was to investigate the therapeutic potential for lymphangiogenesis with **VEGF-C**. We developed a rabbit ear model to simulate human chronic postsurgical lymphatic insufficiency. Successful, sustained surgical ablation of the ear lymphatics was confirmed by water displacement volumetry. After complete healing, the experimental animals (n=8) received a single, s.c. 100 microg dose of

VEGF-C in the operated ear; controls (n=8) received normal saline. Radionuclide lymphoscintigraphy was performed to quantitate lymphatic function. Immunohistochemistry (IHC) was performed 7-8 days following treatment. After **VEGF-C**, there was a quantifiable amelioration of lymphatic function. IHC confirmed a significant increase in lymphatic vascularity, along with reversal of the intense tissue hypercellularity of untreated lymphedema. This study confirms the capacity of a single dose of **VEGF-C** to induce therapeutic lymphangiogenesis in acquired lymphedema. In addition to improving lymphatic function and vascularity, **VEGF-C** can apparently reverse the abnormalities in tissue architecture that accompany chronic lymphatic insufficiency.

L32 ANSWER 51 OF 121 MEDLINE on STN DUPLICATE 24
 2002209188. PubMed ID: 11943725. Vascular endothelial growth factor ligands and receptors that regulate human cytotrophoblast survival are dysregulated in severe preeclampsia and hemolysis, elevated liver enzymes, and low platelets syndrome. Zhou Yan; McMaster Michael; Woo Kirstin; Janatpour Mary; Perry Jean; Karpanen Terhi; **Alitalo Kari**; Damsky Caroline; Fisher Susan J. (Department of Stomatology, University of California San Francisco, San Francisco, California 94143-0512, USA.) The American journal of pathology, (2002 Apr) Vol. 160, No. 4, pp. 1405-23. Journal code: 0370502. ISSN: 0002-9440. Pub. country: United States. Language: English.

AB Human placental development combines elements of tumorigenesis and vasculogenesis. The organ's specialized epithelial cells, termed cytotrophoblasts, invade the uterus where they reside in the interstitial compartment. They also line uterine arteries and veins. During invasion, ectodermally derived cytotrophoblasts undergo pseudovasculogenesis, switching their adhesion molecule repertoire to mimic that of vascular cells. Failures in this transformation accompany the pregnancy complication preeclampsia. Here, we used a combination of in situ and in vitro analyses to characterize the cell's expression of vascular endothelial growth factor (VEGF) family ligands and receptors, key regulators of conventional vasculogenesis and angiogenesis. Cytotrophoblast differentiation and invasion during the first and second trimesters of pregnancy were associated with down-regulation of VEGF receptor (VEGFR)-2. Invasive cytotrophoblasts in early gestation expressed VEGF-A, **VEGF-C**, placental growth factor (PlGF), VEGFR-1, and **VEGFR-3** and, at term, VEGF-A, PlGF, and VEGFR-1. In vitro the cells incorporated VEGF-A into the surrounding extracellular matrix; PlGF was secreted. We also found that cytotrophoblasts responded to the VEGF ligands they produced. Blocking ligand binding significantly decreased their expression of integrin alpha1, an adhesion molecule highly expressed by endovascular cytotrophoblasts, and increased apoptosis. In severe preeclampsia and hemolysis, elevated liver enzymes, and low platelets syndrome, immunolocalization on tissue sections showed that cytotrophoblast VEGF-A and VEGFR-1 staining decreased; staining for PlGF was unaffected. Cytotrophoblast secretion of the soluble form of VEGFR-1 in vitro also increased. Together, the results of this study showed that VEGF family members regulate cytotrophoblast survival and that expression of a subset of family members is dysregulated in severe forms of preeclampsia.

L32 ANSWER 52 OF 121 MEDLINE on STN DUPLICATE 25
 2002345601. PubMed ID: 12087065. Adenoviral **VEGF-C** overexpression induces blood vessel enlargement, tortuosity, and leakiness but no sprouting angiogenesis in the skin or mucous membranes. Saaristo Anne; Veikkola Tanja; Enholm Berndt; Hytonen Maija; Arola Johanna; Pajusola Katri; Turunen Paivi; Jeltsch Michael; Karkkainen Marika J; Kerjaschki Dentscho; Bueler Hansruedi; Yla-Herttuala Seppo; **Alitalo Kari**. (Molecular/Cancer Biology Laboratory and Ludwig Institute for Cancer Research, Biomedicum, University of Helsinki and Helsinki

University Central Hospital, 00014 Helsinki, Finland.) The FASEB journal : official publication of the Federation of American Societies for Experimental Biology, (2002 Jul) Vol. 16, No. 9, pp. 1041-9. Journal code: 8804484. E-ISSN: 1530-6860. Pub. country: United States. Language: English.

- AB Vascular endothelial growth factors (VEGFs) and their receptors (VEGFRs) are important regulators of blood and lymphatic vessel growth and vascular permeability. The **VEGF-C/VEGFR-3** signaling pathway is crucial for lymphangiogenesis, and heterozygous inactivating missense mutations of the **VEGFR-3** gene are associated with hereditary lymphedema. However, **VEGF-C** can have potent effects on blood vessels because its receptor **VEGFR-3** is expressed in certain blood vessels and because the fully processed form of **VEGF-C** also binds to the VEGFR-2 of blood vessels. To characterize the in vivo effects of **VEGF-C** on blood and lymphatic vessels, we have overexpressed **VEGF-C** via adenovirus- and adeno-associated virus-mediated transfection in the skin and respiratory tract of athymic nude mice. This resulted in dose-dependent enlargement and tortuosity of veins, which, along with the collecting lymphatic vessels were found to express VEGFR-2. Expression of angiopoietin 1 blocked the increased leakiness of the blood vessels induced by **VEGF-C** whereas vessel enlargement and lymphangiogenesis were not affected. However, angiogenic sprouting of new blood vessels was not observed in response to AdVEGF-C or AAV-**VEGF-C**. These results show that virally produced **VEGF-C** induces blood vessel changes, including vascular leak, but its angiogenic potency is much reduced compared with VEGF in normal skin.

L32 ANSWER 53 OF 121 MEDLINE on STN DUPLICATE 26
2002454843. PubMed ID: 12213723. Tumor-associated macrophages express lymphatic endothelial growth factors and are related to peritumoral lymphangiogenesis. Schoppmann Sebastian F; Birner Peter; Stockl Johannes; Kalt Romana; Ullrich Robert; Caucig Carola; Kriehuber Ernst; Nagy Katalin; **Alitalo Kari**; Kerjaschki Dentscho. (Department of Pathology, University of Vienna-Allgemeines Krankenhaus, Austria.) The American journal of pathology, (2002 Sep) Vol. 161, No. 3, pp. 947-56. Journal code: 0370502. ISSN: 0002-9440. Pub. country: United States. Language: English.

- AB Formation of lymphatic metastasis is the initial step of generalized spreading of tumor cells and predicts poor clinical prognosis. Lymphatic vessels generally arise within the peritumoral stroma, although the lymphangiopoietic vascular endothelial growth factors (**VEGF**)-**C** and -**D** are produced by tumor cells. In a carefully selected collection of human cervical cancers (stage pT1b1) we demonstrate by quantitative immunohistochemistry and in situ hybridization that density of lymphatic microvessels is significantly increased in peritumoral stroma, and that a subset of stromal cells express large amounts of **VEGF-C** and VEGF-D. The density of cells producing these vascular growth factors correlates with peritumoral inflammatory stroma reaction, lymphatic microvessel density, and indirectly with peritumoral carcinomatous lymphangiosis and frequency of lymph node metastasis. The **VEGF-C**- and VEGF-D-producing stroma cells were identified in situ as a subset of activated tumor-associated macrophages (TAMs) by expression of a panel of macrophage-specific markers, including CD68, CD23, and CD14. These TAMs also expressed the **VEGF-C**- and VEGF-D-specific tyrosine kinase receptor **VEGFR-3**. As TAMs are derived from monocytes in the circulation, a search in peripheral blood for candidate precursors of **VEGFR-3**-expressing TAMs revealed a subfraction of CD14-positive, **VEGFR-3**-expressing monocytes, that, however, failed to express **VEGF-C** and VEGF-D. Only after in vitro incubation with tumor necrosis factor-alpha, lipopolysaccharide, or VEGF-D

did these monocytes start to synthesize **VEGF-C** de novo. In conclusion **VEGF-C**-expressing TAMs play a novel role in peritumoral lymphangiogenesis and subsequent dissemination in human cancer.

L32 ANSWER 54 OF 121 MEDLINE on STN DUPLICATE 27
2002211556. PubMed ID: 11948478. **VEGF-C** induced lymphangiogenesis is associated with lymph node metastasis in orthotopic MCF-7 tumors. Mattila Mirjami M-T; Ruohola Johanna K; Karpanen Terhi; Jackson David G; **Alitalo Kari**; Harkonen Pirkko L. (Department of Anatomy, Institute of Biomedicine and MediCity Research Laboratory, University of Turku, Turku, Finland.) International journal of cancer. Journal international du cancer, (2002 Apr 20) Vol. 98, No. 6, pp. 946-51. Journal code: 0042124. ISSN: 0020-7136. Pub. country: United States. Language: English.

AB The spread of cancer cells to regional lymph nodes through the lymphatic system is the first step in the dissemination of breast cancer. In several human cancers including those of the breast and prostate, the expression of vascular endothelial growth factor C (**VEGF-C**) is associated with lymph node metastasis. Our study was undertaken to evaluate the effect of **VEGF-C** on metastasis of poorly invasive, estrogen dependent human MCF-7 breast cancer cells. MCF-7 breast cancer cells transfected with **VEGF-C** (MCF-7-**VEGF-C**) were grown as tumors in the mammary fat pads of nude mice implanted with subcutaneous estrogen pellets. Tumor lymphangiogenesis and lymph node metastasis were studied immunohistochemically using antibodies against lymphatic vessel hyaluronan receptor -1 (LYVE-1), VEGF receptor-3 (**VEGFR-3**), PECAM-1, pan-cytokeratin and estrogen dependent pS2 protein. Overexpression of **VEGF-C** in transfected MCF-7 cells stimulated in vivo tumor growth in xenotransplanted mice without affecting estrogen responsiveness. The resulting tumors metastasized to the regional lymph nodes in 75% (in 6 mice out of 8, Experiment I) and in 62% (in 5 mice out of 8, Experiment II) of mice bearing orthotopic tumors formed by MCF-7-**VEGF-C** cells whereas no metastases were observed in mice bearing tumors of control vector-transfected MCF-7 cells (MCF-7-Mock). The density of intratumoral and peritumoral lymphatic vessels was increased in tumors derived from MCF-7-**VEGF-C** cells but not MCF-7-Mock cells. Taken together, our results show that **VEGF-C** overexpression stimulates tumor lymphangiogenesis and induces normally poorly metastatic estrogen-dependent MCF-7 tumors to disseminate to local lymph nodes. These data suggest that **VEGF-C** has an important role in lymph node metastasis of breast cancer even at its hormone-dependent early stage.
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L32 ANSWER 55 OF 121 MEDLINE on STN DUPLICATE 28
2002160407. PubMed ID: 11867607. Altered expression patterns of VEGF receptors in human diabetic retina and in experimental VEGF-induced retinopathy in monkey. Witmer Antonella N; Blaauwgeers Harriet G; Weich Herbert A; **Alitalo Kari**; Vrensen Gijs F J M; Schlingemann Reinier O. (Ocular Angiogenesis Group, Department of Ophthalmology, Academic Medical Center, University of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands.) Investigative ophthalmology & visual science, (2002 Mar) Vol. 43, No. 3, pp. 849-57. Journal code: 7703701. ISSN: 0146-0404. Pub. country: United States. Language: English.

AB PURPOSE: The vascular endothelial growth factor (VEGF) family is involved in vascular leakage and angiogenesis in diabetic retinopathy (DR) in the eye, but may also have physiological functions. Based on the hypothesis that differential VEGF receptor (VEGFR) expression in the retina is an important determinant of effects of VEGF, this study was conducted to investigate VEGFR expression in the diabetic retina and in an experimental

monkey model of VEGF-A-induced retinopathy. **METHODS:** In retinas of 27 eyes of diabetic donors, 18 eyes of nondiabetic control donors, and 4 monkey eyes injected with PBS or VEGF-A, expression patterns of VEGFR-1, -2, and -3 in relation to leaky microvessels, as identified by the marker pathologische anatomie Leiden-endothelium (PAL-E) were studied by immunohistochemistry. **RESULTS.** In control human retinas and retinas of PBS-injected monkey eyes, all three VEGFRs were expressed in nonvascular areas, but only VEGFR-1 was constitutively expressed in retinal microvessels. In diabetic eyes, increased microvascular VEGFR-2 expression was found in association with PAL-E expression, whereas microvascular **VEGFR-3** was present in a subset of PAL-E-positive cases. In VEGF-A-injected monkey eyes, VEGFR-1, -2, and -3 and PAL-E were expressed in retinal microvessels. **CONCLUSIONS:** The VEGFR-1, -2, and -3 expression patterns in control retinas suggest physiological functions of VEGFs that do not involve the vasculature. Initial vascular VEGF signaling may act primarily through VEGFR-1. In diabetic eyes, expression of retinal VEGFR-2 and -3 is increased, mainly in leaky microvessels, and VEGF-A induces vascular expression of the VEGF-A receptor VEGFR-2 and the **VEGF-C/D** receptor **VEGFR-3**. These findings indicate a dual role of VEGFs in the physiology and pathophysiology of the retina and suggest that microvascular VEGFR-2 and -3 signaling by VEGFs occurs late in the pathogenesis of DR, possibly initiated by high levels of VEGF-A in established nonproliferative DR.

L32 ANSWER 56 OF 121 MEDLINE on STN DUPLICATE 29
 2002306448. PubMed ID: 12048269. Suppression of tumor lymphangiogenesis and lymph node metastasis by blocking vascular endothelial growth factor receptor 3 signaling. He Yulong; Kozaki Ken-Ichi; Karpanen Terhi; Koshikawa Katsumi; Yla-Herttuala Seppo; Takahashi Takashi; **Alitalo Kari**. (Molecular/Cancer Biology Laboratory and Ludwig Institute for Cancer Research, Haartman Institute and Helsinki University Central Hospital, Biomedicum Helsinki, University of Helsinki, Finland.) Journal of the National Cancer Institute, (2002 Jun 5) Vol. 94, No. 11, pp. 819-25. Journal code: 7503089. ISSN: 0027-8874. Pub. country: United States. Language: English.

AB BACKGROUND: Vascular endothelial growth factor C (**VEGF-C**) stimulates tumor lymphangiogenesis (i.e., formation of lymphatic vessels) and metastasis to regional lymph nodes by interacting with VEGF receptor 3 (**VEGFR-3**). We sought to determine whether inhibiting **VEGFR-3** signaling, and thus tumor lymphangiogenesis, would inhibit tumor metastasis. **METHODS:** We used the highly metastatic human lung cancer cell line NCI-H460-LNM35 (LNM35) and its parental line NCI-H460-N15 (N15) with low metastatic capacity. We inserted genes by transfection and established a stable N15 cell line secreting **VEGF-C** and a LNM35 cell line secreting the soluble fusion protein VEGF receptor 3-immunoglobulin (**VEGFR-3-Ig**, which binds **VEGF-C** and inhibits **VEGFR-3** signaling). Control lines were transfected with mock vectors. Tumor cells were implanted subcutaneously into severe combined immunodeficient mice (n = 6 in each group), and tumors and metastases were examined 6 weeks later. In another approach, recombinant adenoviruses expressing **VEGFR-3-Ig** (AdR3-Ig) or beta-galactosidase (AdLacZ) were injected intravenously into LNM35 tumor-bearing mice (n = 14 and 7, respectively). **RESULTS:** LNM35 cells expressed higher levels of **VEGF-C** RNA and protein than did N15 cells. Xenograft mock vector-transfected LNM35 tumors showed more intratumoral lymphatic vessels (15.3 vessels per grid; 95% confidence interval [CI] = 13.3 to 17.4) and more metastases in draining lymph nodes (12 of 12) than **VEGFR-3-Ig**-transfected LNM35 tumors (4.1 vessels per grid; 95% CI = 3.4 to 4.7; P<.001, two-sided t test; and four lymph nodes with metastases of 12 lymph nodes examined). Lymph node metastasis was also inhibited in AdR3-Ig-treated mice (AdR3-Ig = 0 of 28

lymph nodes; AdLacZ = 11 of 14 lymph nodes). However, metastasis to the lungs occurred in all mice, suggesting that LNM35 cells can also spread via other mechanisms. N15 tumors overexpressing **VEGF-C** contained more lymphatic vessels than vector-transfected tumors but did not have increased metastatic ability. CONCLUSIONS: Lymph node metastasis appears to be regulated by additional factors besides **VEGF-C**. Inhibition of **VEGFR-3** signaling can suppress tumor lymphangiogenesis and metastasis to regional lymph nodes but not to lungs.

L32 ANSWER 57 OF 121 MEDLINE on STN DUPLICATE 30
2002489402. PubMed ID: 12235206. Lymphangiogenic gene therapy with minimal blood vascular side effects. Saaristo Anne; Veikkola Tanja; Tammela Tuomas; Enholm Berndt; Karkkainen Marika J; Pajusola Katri; Bueler Hansruedi; Yla-Herttuala Seppo; **Alitalo Kari**. (Molecular/Cancer Biology Laboratory and Ludwig Institute for Cancer Research, Biomedicum Helsinki, Helsinki University Central Hospital, University of Helsinki, Finland...) The Journal of experimental medicine, (2002 Sep 16) Vol. 196, No. 6, pp. 719-30. Journal code: 2985109R. ISSN: 0022-1007. Pub. country: United States. Language: English.

AB Recent work from many laboratories has demonstrated that the vascular endothelial growth factor-C/VEGF-D/**VEGFR-3** signaling pathway is crucial for lymphangiogenesis, and that mutations of the Vegfr3 gene are associated with hereditary lymphedema. Furthermore, **VEGF-C** gene transfer to the skin of mice with lymphedema induced a regeneration of the cutaneous lymphatic vessel network. However, as is the case with VEGF, high levels of **VEGF-C** cause blood vessel growth and leakiness, resulting in tissue edema. To avoid these blood vascular side effects of **VEGF-C**, we constructed a viral vector for a **VEGFR-3**-specific mutant form of **VEGF-C** (VEGF-C156S) for lymphedema gene therapy. We demonstrate that VEGF-C156S potently induces lymphangiogenesis in transgenic mouse embryos, and when applied via viral gene transfer, in normal and lymphedema mice. Importantly, adenoviral VEGF-C156S lacked the blood vascular side effects of VEGF and **VEGF-C** adenoviruses. In particular, in the lymphedema mice functional cutaneous lymphatic vessels of normal caliber and morphology were detected after long-term expression of VEGF-C156S via an adeno associated virus. These results have important implications for the development of gene therapy for human lymphedema.

L32 ANSWER 58 OF 121 MEDLINE on STN DUPLICATE 31
2002344539. PubMed ID: 12087132. Vascular growth factors and lymphangiogenesis. Jussila Lotta; **Alitalo Kari**. (Molecular/Cancer Biology Laboratory and Ludwig Institute for Cancer Research, and Helsinki University Central Hospital, Biomedicum Helsinki, University of Helsinki, Finland.) Physiological reviews, (2002 Jul) Vol. 82, No. 3, pp. 673-700. Ref: 232. Journal code: 0231714. ISSN: 0031-9333. Pub. country: United States. Language: English.

AB Blood and lymphatic vessels develop in a parallel, but independent manner, and together form the circulatory system allowing the passage of fluid and delivering molecules within the body. Although the lymphatic vessels were discovered already 300 years ago, at the same time as the blood circulation was described, the lymphatic system has remained relatively neglected until recently. This is in part due to the difficulties in recognizing these vessels in tissues because of a lack of specific markers. Over the past few years, several molecules expressed specifically in the lymphatic endothelial cells have been characterized, and knowledge about the lymphatic system has started to accumulate again. The vascular endothelial growth factor (VEGF) family of growth factors and receptors is involved in the development and growth of the vascular endothelial system. Two of its family members, **VEGF-C** and VEGF-D, regulate the lymphatic endothelial cells via their receptor

VEGFR-3. With the aid of these molecules, lymphatic endothelial cells can be isolated and cultured, allowing detailed studies of the molecular properties of these cells. Also the role of the lymphatic endothelium in immune responses and certain pathological conditions can be studied in more detail, as the blood and lymphatic vessels seem to be involved in many diseases in a coordinated manner. Discoveries made so far will be helpful in the diagnosis of certain vascular tumors, in the design of specific treatments for lymphedema, and in the prevention of metastatic tumor spread via the lymphatic system.

L32 ANSWER 59 OF 121 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

2002:589932 The Genuine Article (R) Number: 570WR. The transcription factor Prox1 is a marker for lymphatic endothelial cells in normal and diseased human tissues. Wilting J (Reprint); Papoutsis M; Christ B; Nicolaides K H; von Kaisenberg C S; Borges J; Stark G B; **Alitalo K**; Tomarev S I; Niemeyer C; Rossler J. Univ Freiburg, Inst Anat, Albertstr 17, D-79104 Freiburg, Germany (Reprint); Univ Freiburg, Inst Anat, D-79104 Freiburg, Germany; Univ London Kings Coll Hosp, Harris Birthright Res Ctr Fetal Med, London, England; Univ Kiel, Frauenklinik, D-24105 Kiel, Germany; Univ Freiburg, Abt Plast & Handchirurg, D-79104 Freiburg, Germany; Univ Helsinki, Mol & Canc Biol Lab, FIN-00014 Helsinki, Finland; NEI, Mol & Dev Biol Lab, NIH, Bethesda, MD 20892 USA; Univ Freiburg, Zentrum Kinderheilkunde & Jugendmed, D-79104 Freiburg, Germany. FASEB JOURNAL (JUN 2002) Vol. 16, No. 8, pp. U515-U530. ISSN: 0892-6638. Publisher: FEDERATION AMER SOC EXP BIOL, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3998 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Detection of lymphatic endothelial cells (LECs) has been problematic because of the lack of specific markers. The homeobox transcription factor Prox1 is expressed in LECs of murine and avian embryos. We have studied expression of Prox1 in human tissues with immunofluorescence. In 19-wk-old human fetuses, Prox1 and vascular endothelial growth factor receptor-3 (**VEGFR-3**) are coexpressed in LECs of lymphatic trunks and lymphatic capillaries. Prox1 is located in the nucleus, and its expression is mutually exclusive with that of the blood vascular marker PAL-E. Prox1 is a constitutive marker of LECs and is found in tissues of healthy adults and lymphedema patients. Blood vascular endothelial cells (BECs) of hemangiomas express CD31 and CD34, but not Prox1. A subset of these cells is positive for **VEGFR-3**. Lymphatics in the periphery of hemangiomas express Prox1 and CD31, but not CD34. In lymphangiomas, LECs express Prox1, CD31, and **VEGFR-3**, but rarely CD34. In the stroma, spindle-shaped CD34-positive cells are present. We show that Prox1 is a reliable marker for LECs in normal and pathologic human tissues, coexpressed with **VEGFR-3** and CD31. **VEGFR-3** and CD34 are less reliable markers for LECs and BECs, respectively, because exceptions from their normal expression patterns are found in pathologic tissues.

L32 ANSWER 60 OF 121 MEDLINE on STN DUPLICATE 32

2002344080. PubMed ID: 12086857. Molecular mechanisms of lymphangiogenesis in health and disease. **Alitalo Kari**; Carmeliet Peter. (Molecular/Cancer Biology Laboratory, Biomedicum Helsinki, Haartman Institute and Helsinki University Central Hospital, POB 63 (Haartmaninkatu 8), 00014 University of Helsinki, Finland.) Cancer cell, (2002 Apr) Vol. 1, No. 3, pp. 219-27. Ref: 73. Journal code: 101130617. ISSN: 1535-6108. Pub. country: United States. Language: English.

AB Studies of the last decades have revealed the importance of angiogenesis for normal growth and for the pathogenesis of numerous diseases. Much less studied is lymphangiogenesis, the growth of lymphatic vessels, which drain extravasated fluid, proteins, and cells and transport them back to the venous circulation. Nonetheless, insufficient lymphangiogenesis

causes incapacitating lymphedema, while lymphatic growth around tumors may facilitate metastatic spread of malignant cells that ultimately kill the patient. The recent discovery of the key lymphangiogenic factors **VEGF-C** and **VEGF-D** and their receptor **VEGFR-3** has allowed novel insights into how the lymphatic vessels and blood vessels coordinately grow and affect human disease. In addition, these studies have opened novel diagnostic and therapeutic avenues for the treatment of lymphedema and metastasis. This overview highlights the recent insights and developments in the field of lymphatic vascular research.

L32 ANSWER 61 OF 121 CAPLUS COPYRIGHT 2006 ACS on STN

2003:535307 Document No. 140:214419 Molecular mechanisms of lymphangiogenesis. Makinen, T.; **Alitalo, K.** (Molecular/Cancer Biology Laboratory and Ludwig Institute for Cancer Research, Haartman Institute and Helsinki University Hospital, University of Helsinki, Helsinki, 00014, Finland). Cold Spring Harbor Symposia on Quantitative Biology, 67, 189-196 (English) 2002. CODEN: CSHSAZ. ISSN: 0091-7451. Publisher: Cold Spring Harbor Laboratory Press.

AB A review on the mol. mechanisms regulating the development and growth of the lymphatic vessels. Two members of the vascular endothelial growth factor (VEGF) family, **VEGF-C** and **VEGF-D**, have been shown to stimulate the growth of lymphatic vessels via the lymphatic endothelial cell receptor, **VEGFR-3**. This receptor mediates signals for the survival, migration, and proliferation of lymphatic endothelial cells, and these signals are essential for the normal development of the lymphatic vasculature. Blocking of **VEGFR-3** signaling inhibits specifically fetal lymphangiogenesis without affecting blood vessel development, indicating that distinct mol. mechanisms control the development of the two vascular systems.

L32 ANSWER 62 OF 121 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

2002:501357 Document No.: PREV200200501357. Growth factor regulation of angiogenesis, lymph-angiogenesis and metastasis. **Alitalo, Kari** [Reprint author]; et al. Molecular/Cancer Biology Laboratory, Biomedicum Helsinki, University of Helsinki, Helsinki, Finland. Pathology Research and Practice, (2002) Vol. 198, No. 3, pp. 173. print. Meeting Info.: 86th Meeting of the German Society of Pathology. Vienna, Austria. April 03-06, 2002. CODEN: PARPDS. ISSN: 0344-0338. Language: English.

L32 ANSWER 63 OF 121 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

2002:947688 The Genuine Article (R) Number: 614BN. Therapeutic lymphangiogenesis with human recombinant **VEGF-C**. Szuba A; Skobe M; Karkkainen M J; Shin W S; Beynet D P; Rockson N B (Reprint); Dakhil N; Spilman S; Goris M L; Strauss H W; Quertermous T; **Alitalo K**; Rockson S G. Stanford Univ, Sch Med, Div Cardiovasc Med, Falk Cardiovasc Res Ctr, Stanford, CA 94305 USA (Reprint); Derald H Ruttenberg Canc Ctr, Mt Sinai Sch Med, New York, NY USA; Univ Helsinki, Haartman Inst, Helsinki, Finland. FASEB JOURNAL (OCT 2002) Vol. 16, No. 12, pp. U114-U130. ISSN: 0892-6638. Publisher: FEDERATION AMER SOC EXP BIOL, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3998 USA. Language: English. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Chronic regional impairments of the lymphatic circulation often lead to striking architectural abnormalities in the lymphedematous tissues. Lymphedema is a common, disabling disease that currently lacks a cure. Vascular endothelial growth factors C and D mediate lymphangiogenesis through the **VEGFR-3** receptor on lymphatic endothelia. The purpose of this study was to investigate the therapeutic potential for lymphangiogenesis with **VEGF-C**. We developed a rabbit

ear model to simulate human chronic postsurgical lymphatic insufficiency. Successful, sustained surgical ablation of the ear lymphatics was confirmed by water displacement volumetry. After complete healing, the experimental animals (n=8) received a single, s.c. 100 mug dose of **VEGF-C** in the operated ear; controls (n=8) received normal saline. Radionuclide lymphoscintigraphy was performed to quantitate lymphatic function. Immunohistochemistry (IHC) was performed 7-8 days following treatment. After **VEGF-C**, there was a quantifiable amelioration of lymphatic function. IHC confirmed a significant increase in lymphatic vascularity, along with reversal of the intense tissue hypercellularity of untreated lymphedema. This study confirms the capacity of a single dose of **VEGF-C** to induce therapeutic lymphangiogenesis in acquired lymphedema. In addition to improving lymphatic function and vascularity, **VEGF-C** can apparently reverse the abnormalities in tissue architecture that accompany chronic lymphatic insufficiency.

- L32 ANSWER 64 OF 121 MEDLINE on STN DUPLICATE 33
 2003064740. PubMed ID: 12543720. Insights into the molecular pathogenesis and targeted treatment of lymphedema. Saaristo Anne; Karkkainen Marika J; **Alitalo Kari**. (Molecular/Cancer Biology Laboratory, Biomedicum, University of Helsinki, Helsinki, Finland.) Annals of the New York Academy of Sciences, (2002 Dec) Vol. 979, pp. 94-110. Ref: 78. Journal code: 7506858. ISSN: 0077-8923. Pub. country: United States. Language: English.
- AB Abnormal function of the lymphatic vessels is associated with a variety of diseases, such as tumor metastasis and lymphedema. The development of strategies for local and controlled induction or inhibition of lymphangiogenesis would thus be of major importance for the treatment of such diseases. Two growth factors, vascular endothelial growth factor C (**VEGF-C**) and D (**VEGF-D**), have been found to be important in the proper formation and maintenance of the lymphatic network, through their receptor **VEGFR-3**. In patients with lymphedema, heterozygous inactivation of **VEGFR-3** leads to primary lymphedema due to defective lymphatic drainage in the limbs. We have shown that **VEGF-C** gene transfer to the skin of mice with lymphedema induces regeneration of the cutaneous lymphatic vessel network. However, as is the case with VEGF, high levels of **VEGF-C** cause blood vessel growth and leakiness, resulting in tissue edema. Strategies to avoid these side-effects have also been developed. This new field of research has important implications for the development of new therapies for human lymphedema.
- L32 ANSWER 65 OF 121 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
 2002:455381 Document No.: PREV200200455381. Growth factor regulation of angiogenesis, lymphangiogenesis and metastasis. **Alitalo, K**. [Reprint author]. Molecular/Cancer Biology Laboratory, Biomedicum Helsinki, University of Helsinki, Helsinki, Finland. European Journal of Cancer, (March, 2002) Vol. 38, No. Supplement 3, pp. S90. print. Meeting Info.: 3rd European Breast Cancer Conference. Barcelona, Spain. March 19-23, 2002. CODEN: EJCAEL. ISSN: 0959-8049. Language: English.
- L32 ANSWER 66 OF 121 MEDLINE on STN DUPLICATE 34
 2002094769. PubMed ID: 11824969. Vascular endothelial growth factors C and D and their VEGFR-2 and 3 receptors in blood and lymphatic vessels in healthy and arthritic synovium. Paavonen Karri; Mandelin Jami; Partanen Taina; Jussila Lotta; Li Tian-Fang; Ristimaki Ari; **Alitalo Kari**; Konttinen Yrjo T. (Molecular/Cancer Biology Laboratory, Biomedicum Helsinki, University of Helsinki, Finland.) The Journal of rheumatology, (2002 Jan) Vol. 29, No. 1, pp. 39-45. Journal code: 7501984. ISSN: 0315-162X. Pub. country: Canada. Language: English.

AB OBJECTIVE: To localize vascular endothelial growth factor C (**VEGF-C**) and VEGF-D in synovial specimens in relation to their VEGFR-2 and **VEGFR-3** receptors in blood and lymphatic vessels. METHODS: Immunohistochemical staining and messenger RNA analysis from control and arthritic synovial membrane specimens. RESULTS: Quantitative RT-PCR disclosed that **VEGF-C** mRNA copy numbers were higher than VEGF-D mRNA copy numbers in the rheumatoid arthritis (RA), osteoarthritis, and control patient groups studied ($p < 0.01$). Immunohistochemical staining localized **VEGF-C** to synovial lining cell layer, pericytes, and smooth muscle cells of blood vessels. The number of **VEGF-C** positive cells was increased in the synovial lining of ankylosing spondylitis (AS) and RA compared to control synovium. However, in contrast to control synovial lining, little if any VEGF-D was detected in AS or RA synovial lining. VEGFR-2 expressing stromal blood vessels, also positive for the vascular endothelial marker PAL-E and the basement membrane marker laminin, were more abundant in RA and AS than in controls. Interestingly, the lymphatic endothelial receptor **VEGFR-3** was also expressed in most synovial vessels, especially in the sublining capillaries and venules. CONCLUSION: **VEGF-C** is strongly expressed in the hypertrophic synovial lining of arthritic joints, whereas VEGF-D expression is very low in AS and RA. The expression of **VEGF-C** and VEGF-D in pericytes and smooth muscle cells suggests that these factors may have a role in maintaining vascular homeostasis. The VEGF receptors VEGFR-2 and **VEGFR-3** are present in most of the sublining blood vessels. The expression of the lymphatic marker **VEGFR-3** in the sublining blood vessels may relate to fluid filtration and/or fenestrations. The relatively few lymphatic vessels along with increased vascular permeability in RA may contribute to the development of tissue edema and joint stiffness.

L32 ANSWER 67 OF 121 MEDLINE on STN DUPLICATE 35
2002233233. PubMed ID: 11969367. Lymphatic endothelial regulation, lymphoedema, and lymph node metastasis. Karkkainen Marika J; **Alitalo Kari**. (Molecular/Cancer Biology Laboratory and Ludwig Institute for Cancer Research, Haartman Institute, Biomedicum Helsinki, Helsinki University Hospital and University of Helsinki, 00014 Helsinki, Finland.. Marika.Karkkainen@Helsinki.Fi) . Seminars in cell & developmental biology, (2002 Feb) Vol. 13, No. 1, pp. 9-18. Ref: 100. Journal code: 9607332. ISSN: 1084-9521. Pub. country: England: United Kingdom. Language: English.

AB Vascular endothelial growth factor receptor-3 (**VEGFR-3**) mediates lymphatic endothelial cell (LEC) growth, migration, and survival by binding **VEGF-C** and VEGF-D. Recent studies have revealed new regulators of the lymphatic endothelium, such as the transcription factor Prox1, and the cell surface proteins podoplanin and lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1). Furthermore, the isolation of LECs now allows detailed molecular studies of the factors regulating the lymphatic vasculature. These studies are aimed at targeting the lymphatic vasculature in the treatment of various diseases, such as tumour metastasis and lymphoedema.
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L32 ANSWER 68 OF 121 MEDLINE on STN DUPLICATE 36
2002054196. PubMed ID: 11780131. Lymphatic endothelium: a new frontier of metastasis research. Karkkainen Marika J; Makinen Taija; **Alitalo Kari**. Nature cell biology, (2002 Jan) Vol. 4, No. 1, pp. E2-5. Ref: 41. Journal code: 100890575. ISSN: 1465-7392. Pub. country: England: United Kingdom. Language: English.

AB The vascular endothelium is a dynamic tissue with many active functions. Until recently, endothelial cell (EC) biology studies have used cultured ECs from various organs; these cell lines are considered representative of the blood vascular endothelium. Very few lymphatic EC lines have been available, and these were derived from lymphatic tumours or large

collecting lymphatic ducts. In the past, lymphatic vessels were defined largely by the lack of erythrocytes in their lumen, a lack of junctional complexes and the lack of a well-defined basement membrane. Now that lymphatic-specific vascular endothelial growth factors (**VEGF-C** and **VEGF-D**) and molecular cell surface markers such as the **VEGFR-3** receptor have been identified, this definition needs to be updated. Recent developments have highlighted the importance of lymphatic ECs, and they could become the next focus for angiogenesis and metastasis research.

L32 ANSWER 69 OF 121 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN

2002029739 EMBASE Lymphatic endothelium: A new frontier of metastasis research. Karkkainen M.J.; Makinen T.; **Alitalo K.** Nature Cell Biology Vol. 4, No. 1, pp. E2-E5 2002.

Refs: 41.

ISSN: 1465-7392. CODEN: NCBIFN

Pub. Country: United Kingdom. Language: English. Summary Language: English.

Entered STN: 20020131. Last Updated on STN: 20020131

AB The vascular endothelium is a dynamic tissue with many active functions. Until recently, endothelial cell (EC) biology studies have used cultured ECs from various organs; these cell lines are considered representative of the blood vascular endothelium. Very few lymphatic EC lines have been available, and these were derived from lymphatic tumours or large collecting lymphatic ducts. In the past, lymphatic vessels were defined largely by the lack of erythrocytes in their lumen, a lack of junctional complexes and the lack of a well-defined basement membrane. Now that lymphatic-specific vascular endothelial growth factors (**VEGF-C** and **VEGF-D**) and molecular cell surface markers such as the **VEGFR-3** receptor have been identified, this definition needs to be updated. Recent developments have highlighted the importance of lymphatic ECs, and they could become the next focus for angiogenesis and metastasis research.

L32 ANSWER 70 OF 121 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

2001:945005 The Genuine Article (R) Number: 494QQ. Multiple forms of mouse vascular endothelial growth factor-D are generated by RNA splicing and proteolysis. Baldwin M E; Roufail S; Halford M M; **Alitalo K**; Stacker S A; Achen M G (Reprint). Royal Melbourne Hosp, Ludwig Inst Canc Res, POB 2008, Melbourne, Vic 3050, Australia (Reprint); Royal Melbourne Hosp, Ludwig Inst Canc Res, Melbourne, Vic 3050, Australia; Univ Helsinki, Mol Canc Biol Lab, FIN-00014 Helsinki, Finland. JOURNAL OF BIOLOGICAL CHEMISTRY (23 NOV 2001) Vol. 276, No. 47, pp. 44307-44314. ISSN: 0021-9258 . Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The secreted glycoprotein vascular endothelial growth factor-D (**VEGF-D**) is angiogenic, lymphangiogenic, and promotes metastatic spread of tumor cells via lymphatic vessels. **VEGF-D** consists of a receptor-binding domain (**VEGF** homology domain) and N- and C terminal propeptides. Proteolytic processing produces numerous forms of human **VEGF-D**, including fully processed derivatives (containing only the **VEGF** homology domain), partially processed, and unprocessed derivatives. Proteolysis is essential to generate human **VEGF-D** that binds the angiogenic receptor **VEGF** receptor-2 (**VEGFR-2**) and the lymphangiogenic receptor **VEGFR-3** with high affinity. Here, we report that alternative use of an RNA splice donor site in exon 6 of the mouse **VEGF-D** gene produces two different protein isoforms, **VEGF-D-358** and **VEGF-D-326**, with distinct C termini. The two isoforms were both expressed in all adult mouse tissues and embryonic stages of development analyzed. Both isoforms are proteolytically processed in a similar fashion to human **VEGF-D** to generate

a range of secreted derivatives and bind and cross-link **VEGFR-3** with similar potency. The isoforms are differently glycosylated when expressed in vitro. This study demonstrates that RNA splicing, protein glycosylation, and proteolysis are mechanisms for generating structural diversity of mouse VEGF-D.

L32 ANSWER 71 OF 121 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

2001:466906 The Genuine Article (R) Number: 439BM. The specificity of receptor binding by vascular endothelial growth factor-D is different in mouse and man. Baldwin M E; Catimel B; Nice E C; Roufail S; Hall N E; Stenvers K L; Karkkainen M J; **Alitalo K**; Stacker S A; Achen M G (Reprint). Royal Melbourne Hosp, Ludwig Inst Canc Res, POB 2008, Melbourne, Vic 3050, Australia (Reprint); Royal Melbourne Hosp, Ludwig Inst Canc Res, Melbourne, Vic 3050, Australia; Univ Helsinki Hosp, FIN-00014 Helsinki, Finland; Haartman Inst, Mol Canc Biol Lab, FIN-00014 Helsinki, Finland. JOURNAL OF BIOLOGICAL CHEMISTRY (1 JUN 2001) Vol. 276, No. 22, pp. 19166-19171. ISSN: 0021-9258. Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3996 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Human vascular endothelial growth factor-D (VEGF-D) binds and activates VEGFR-2 and **VEGFR-3**, receptors expressed on vascular and lymphatic endothelial cells. As VEGFR-2 signals for angiogenesis and **VEGFR-3** is thought to signal for lymphangiogenesis, it was proposed that VEGF-D stimulates growth of blood vessels and lymphatic vessels into regions of embryos and tumors. Here we report the unexpected finding that mouse VEGF-D fails to bind mouse VEGFR-2 but binds and cross-links **VEGFR-3** as demonstrated by biosensor analysis with immobilized receptor domains and bioassays of VEGFR-2 and **VEGFR-3** cross-linking. Mutation of amino acids in mouse VEGF-D to those in the human homologue indicated that residues important for the VEGFR-2 interaction are clustered at, or are near, the predicted receptor-binding surface. Coordinated expression of VEGF-D and **VEGFR-3** in mouse embryos was detected in the developing skin where the VEGF-D gene was expressed in a layer of cells beneath the developing epidermis and **VEGFR-3** was localized on a network of vessels immediately beneath the VEGF-D-positive cells. This suggests that VEGF-D and **VEGFR-3** may play a role in establishing vessels of the skin by a paracrine mechanism. Our study of receptor specificity suggests that VEGF-D may have different biological functions in mouse and man.

L32 ANSWER 72 OF 121 MEDLINE on STN DUPLICATE 37

2001635536. PubMed ID: 11592985. A model for gene therapy of human hereditary lymphedema. Karkkainen M J; Saaristo A; Jussila L; Karila K A; Lawrence E C; Pajusola K; Bueler H; Eichmann A; Kauppinen R; Kettunen M I; Yla-Herttuala S; Finegold D N; Ferrell R E; **Alitalo K**. (Molecular/Cancer Biology Laboratory and Ludwig Institute for Cancer Research, Haartman Institute and Helsinki University Hospital, Biomedicum Helsinki, University of Helsinki, P.O.B. 63 (Haartmaninkatu 8), 00014 Helsinki, Finland.) Proceedings of the National Academy of Sciences of the United States of America, (2001 Oct 23) Vol. 98, No. 22, pp. 12677-82. Electronic Publication: 2001-10-09. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Primary human lymphedema (Milroy's disease), characterized by a chronic and disfiguring swelling of the extremities, is associated with heterozygous inactivating missense mutations of the gene encoding vascular endothelial growth factor C/D receptor (**VEGFR-3**). Here, we describe a mouse model and a possible treatment for primary lymphedema. Like the human patients, the lymphedema (Chy) mice have an inactivating Vegfr3 mutation in their germ line, and swelling of the limbs

because of hypoplastic cutaneous, but not visceral, lymphatic vessels. Neuropilin (NRP)-2 bound **VEGF-C** and was expressed in the visceral, but not in the cutaneous, lymphatic endothelia, suggesting that it may participate in the pathogenesis of lymphedema. By using virus-mediated **VEGF-C** gene therapy, we were able to generate functional lymphatic vessels in the lymphedema mice. Our results suggest that growth factor gene therapy is applicable to human lymphedema and provide a paradigm for other diseases associated with mutant receptors.

- L32 ANSWER 73 OF 121 MEDLINE on STN DUPLICATE 38
 2001509463. PubMed ID: 11532940. Isolated lymphatic endothelial cells transduce growth, survival and migratory signals via the **VEGF-C/D** receptor **VEGFR-3**. Makinen T; Veikkola T; Mustjoki S; Karpanen T; Catimel B; Nice E C; Wise L; Mercer A; Kowalski H; Kerjaschki D; Stacker S A; Achen M G; **Alitalo K**. (Molecular/Cancer Biology Laboratory and Ludwig Institute for Cancer Research, and Helsinki University Hospital, Biomedicum Helsinki, University of Helsinki, FIN-00014 Helsinki, Finland.) The EMBO journal, (2001 Sep 3) Vol. 20, No. 17, pp. 4762-73. Journal code: 8208664. ISSN: 0261-4189. Pub. country: England: United Kingdom. Language: English.
- AB Vascular endothelial growth factor receptor-3 (**VEGFR-3** /Flt4) binds two known members of the VEGF ligand family, **VEGF-C** and **VEGF-D**, and has a critical function in the remodelling of the primary capillary vasculature of midgestation embryos. Later during development, **VEGFR-3** regulates the growth and maintenance of the lymphatic vessels. In the present study, we have isolated and cultured stable lineages of blood vascular and lymphatic endothelial cells from human primary microvascular endothelium by using antibodies against the extracellular domain of **VEGFR-3**. We show that **VEGFR-3** stimulation alone protects the lymphatic endothelial cells from serum deprivation-induced apoptosis and induces their growth and migration. At least some of these signals are transduced via a protein kinase C-dependent activation of the p42/p44 MAPK signalling cascade and via a wortmannin-sensitive induction of Akt phosphorylation. These results define the critical role of **VEGF-C/VEGFR-3** signalling in the growth and survival of lymphatic endothelial cells. The culture of isolated lymphatic endothelial cells should now allow further studies of the molecular properties of these cells.

- L32 ANSWER 74 OF 121 MEDLINE on STN DUPLICATE 39
 2001189626. PubMed ID: 11280723. Vascular endothelial growth factor C promotes tumor lymphangiogenesis and intralymphatic tumor growth. Karpanen T; Egeblad M; Karkkainen M J; **Kubo H**; Yla-Herttuala S; Jaattela M; **Alitalo K**. (Molecular/Cancer Biology Laboratory, Haartman Institute and Ludwig Institute for Cancer Research, University of Helsinki, Finland.) Cancer research, (2001 Mar 1) Vol. 61, No. 5, pp. 1786-90. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.
- AB Many solid tumors produce vascular endothelial growth factor C (**VEGF-C**), and its receptor, **VEGFR-3**, is expressed in tumor blood vessels. To study the role of **VEGF-C** in tumorigenesis, we implanted MCF-7 human breast carcinoma cells overexpressing recombinant **VEGF-C** orthotopically into severe combined immunodeficient mice. **VEGF-C** increased tumor growth, but unlike VEGF, it had little effect on tumor angiogenesis. Instead, **VEGF-C** strongly promoted the growth of tumor-associated lymphatic vessels, which in the tumor periphery were commonly infiltrated with the tumor cells. These effects of **VEGF-C** were inhibited by a soluble **VEGFR-3** fusion protein. Our data suggest that **VEGF-C** facilitates tumor metastasis via the lymphatic vessels and that tumor

spread can be inhibited by blocking the interaction between **VEGF-C** and its receptor.

- L32 ANSWER 75 OF 121 MEDLINE on STN DUPLICATE 40
2001216875. PubMed ID: 11250889. Signalling via vascular endothelial growth factor receptor-3 is sufficient for lymphangiogenesis in transgenic mice. Veikkola T; Jussila L; Makinen T; Karpanen T; Jeltsch M; Petrova T V; **Kubo H**; Thurston G; McDonald D M; Achen M G; Stacker S A; **Alitalo K**. (Molecular/Cancer Biology Laboratory and Ludwig Institute for Cancer Research, Haartman Institute, University of Helsinki, PO Box 21 (Haartmaninkatu 3), 00014 Helsinki, Finland.) The EMBO journal, (2001 Mar 15) Vol. 20, No. 6, pp. 1223-31. Journal code: 8208664. ISSN: 0261-4189. Pub. country: England: United Kingdom. Language: English.
- AB Vascular endothelial growth factor receptor-3 (**VEGFR-3**) has an essential role in the development of embryonic blood vessels; however, after midgestation its expression becomes restricted mainly to the developing lymphatic vessels. The **VEGFR-3** ligand **VEGF-C** stimulates lymphangiogenesis in transgenic mice and in chick chorioallantoic membrane. As **VEGF-C** also binds **VEGFR-2**, which is expressed in lymphatic endothelia, it is not clear which receptors are responsible for the lymphangiogenic effects of **VEGF-C**. **VEGF-D**, which binds to the same receptors, has been reported to induce angiogenesis, but its lymphangiogenic potential is not known. In order to define the lymphangiogenic signalling pathway we have created transgenic mice overexpressing a **VEGFR-3**-specific mutant of **VEGF-C** (**VEGF-C156S**) or **VEGF-D** in epidermal keratinocytes under the keratin 14 promoter. Both transgenes induced the growth of lymphatic vessels in the skin, whereas the blood vessel architecture was not affected. Evidence was also obtained that these growth factors act in a paracrine manner in vivo. These results demonstrate that stimulation of the **VEGFR-3** signal transduction pathway is sufficient to induce specifically lymphangiogenesis in vivo.
- L32 ANSWER 76 OF 121 MEDLINE on STN DUPLICATE 41
2001265781. PubMed ID: 11282897. Adenoviral expression of vascular endothelial growth factor-C induces lymphangiogenesis in the skin. Enholm B; Karpanen T; Jeltsch M; **Kubo H**; Stenback F; Prevo R; Jackson D G; Yla-Herttuala S; **Alitalo K**. (Molecular/Cancer Biology Laboratory and Ludwig Institute for Cancer Research, Haartman Institute, University of Helsinki, Finland.) Circulation research, (2001 Mar 30) Vol. 88, No. 6, pp. 623-9. Journal code: 0047103. E-ISSN: 1524-4571. Pub. country: United States. Language: English.
- AB The growth of blood and lymphatic vasculature is mediated in part by secreted polypeptides of the vascular endothelial growth factor (VEGF) family. The prototype VEGF binds VEGF receptor (**VEGFR**)-1 and **VEGFR-2** and is angiogenic, whereas **VEGF-C**, which binds to **VEGFR-2** and **VEGFR-3**, is either angiogenic or lymphangiogenic in different assays. We used an adenoviral gene transfer approach to compare the effects of these growth factors in adult mice. Recombinant adenoviruses encoding human **VEGF-C** or VEGF were injected subcutaneously into C57Bl6 mice or into the ears of nude mice. Immunohistochemical analysis showed that **VEGF-C** upregulated **VEGFR-2** and **VEGFR-3** expression and VEGF upregulated **VEGFR-2** expression at 4 days after injection. After 2 weeks, histochemical and immunohistochemical analysis, including staining for the lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1), the vascular endothelial marker platelet-endothelial cell adhesion molecule-1 (PECAM-1), and the proliferating cell nuclear antigen (PCNA) revealed that **VEGF-C** induced mainly lymphangiogenesis in contrast to VEGF, which induced only angiogenesis. These results have significant implications in the planning of gene therapy using these growth factors.

L32 ANSWER 77 OF 121 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

2001:481038 Document No.: PREV200100481038. Lymphangiogenesis in melanoma, squamous cell carcinoma and breast carcinoma. Skobe, M. [Reprint author]; Hawighorst, T. [Reprint author]; **Alitalo, K.**; Jackson, D.; Detmar, M. [Reprint author]. Dermatology, Harvard Medical School and Massachusetts General Hospital, Charlestown, MA, USA. Journal of Investigative Dermatology, (August, 2001) Vol. 117, No. 2, pp. 392. print. Meeting Info.: 62nd Annual Meeting of the Society for Investigative Dermatology. Washington, DC, USA. May 09-12, 2001. CODEN: JIDEAE. ISSN: 0022-202X. Language: English.

L32 ANSWER 78 OF 121 CAPLUS COPYRIGHT 2006 ACS on STN

2002:663136 Document No. 137:367312 **VEGF-C**/VEGFRs and cancer metastasis. Yonemura, Yutaka; Endou, Yoshio; Sasaki, Takuma; Sugiyama, Kazuo; Yamashima, Tetumouri; Partanen, Taina; **Alitalo, Kari** (Second Department of Surgery, School of Medicine, Kanazawa University, Kanazawa, 920, Japan). Cancer Metastasis--Biology and Treatment, 2(Growth Factors and Their Receptors in Cancer Metastasis), 223-239 (English) 2001. CODEN: CMTACZ. Publisher: Kluwer Academic Publishers.

AB A review. Vascular endothelial growth factor C (**VEGF-C**) is the only factor known causing lymphangiogenesis. We report herein the review of recent exptl. studies on VEGF family and their receptors and the mol. mechanisms of the lymphangiogenesis in cancer. According to our study, **VEGF-C** is a potent stimulator in not only the angiogenesis but also the lymphangiogenesis on the chick chorioallantoic membrane. In the clin. specimens from gastric cancer, there is an intimate relationship between the VEGF receptor-3/**VEGF-C** tissue status and lymphangiogenesis. RT-PCR and immunohistol. examns. demonstrated that **VEGF-C** was mainly produced from cancer cells and that **VEGFR-3** expression was restricted in the endothelial cells of lymphatic vessels. **VEGF-C** and **VEGFR-3** mRNA expression were pos. correlated in primary gastric cancers and the number of **VEGFR-3** pos. lymphatic vessels in **VEGF-C** mRNA pos. tumor was significantly larger than that in **VEGF-C** neg. tumors. The number of such vessels in tumor stroma was closely related to the grade of lymphatic invasion of gastric cancer. Accordingly, we conclude that **VEGF-C** may induce the lymphatic neogenesis in the stroma of primary gastric cancer. In these circumstances, cancer cells can easily migrate into the lymphatic vessels, because of the increase of the contact point of cancer cells with lymphatic vessels.

L32 ANSWER 79 OF 121 MEDLINE on STN

DUPLICATE 42

2001212645. PubMed ID: 11175851. Inhibition of lymphangiogenesis with resulting lymphedema in transgenic mice expressing soluble VEGF receptor-3. Makinen T; Jussila I; Veikkola T; Karpanen T; Kettunen M I; Pulkkanen K J; Kauppinen R; Jackson D G; **Kubo H**; Nishikawa S; Yla-Herttuala S; **Alitalo K.** (Molecular/Cancer Biology Laboratory and Ludwig Institute for Cancer Research, Haartman Institute, University of Helsinki, Helsinki, Finland.) Nature medicine, (2001 Feb) Vol. 7, No. 2, pp. 199-205. Journal code: 9502015. ISSN: 1078-8956. Pub. country: United States. Language: English.

AB The lymphatic vasculature transports extravasated tissue fluid, macromolecules and cells back into the blood circulation. Recent reports have focused on the molecular mechanisms regulating the lymphatic vessels. Vascular endothelial growth factor (**VEGF**)-C and VEGF-D have been shown to stimulate lymphangiogenesis and their receptor, **VEGFR-3**, has been linked to human hereditary lymphedema. Here we show that a soluble form of **VEGFR-3** is a potent inhibitor of **VEGF-C**/VEGF-D signaling, and when

expressed in the skin of transgenic mice, it inhibits fetal lymphangiogenesis and induces a regression of already formed lymphatic vessels, though the blood vasculature remains normal. Transgenic mice develop a lymphedema-like phenotype characterized by swelling of feet, edema and dermal fibrosis. They survive the neonatal period in spite of a virtually complete lack of lymphatic vessels in several tissues, and later show regeneration of the lymphatic vasculature, indicating that induction of lymphatic regeneration may also be possible in humans.

L32 ANSWER 80 OF 121 CAPLUS COPYRIGHT 2006 ACS on STN

2001:695302 Document No. 135:355978 Lymphatic vessels as targets of tumor therapy?. Karpanen, Terhi; **Alitalo, Kari** (Molecular/Cancer Biology Laboratory and Ludwig Institute for Cancer Research, Haartman Institute, Biomedicum Helsinki, University of Helsinki, Helsinki, 00014, Finland). Journal of Experimental Medicine, 194(6), F37-F42 (English) 2001. CODEN: JEMEAV. ISSN: 0022-1007. Publisher: Rockefeller University Press.

AB A review, with 50 refs., on the mechanisms of tumor therapy through the lymphatic vessels. Two members of the vascular endothelial growth factor (VEGF) family, **VEGF-C** and VEGF-D, were associated with lymphangiogenesis. **VEGF-C** expression was detected in about half of human cancers analyzed. Several studies on the correlation of lymphatic vessels with the VEGF family, specifically **VEGF-C** are discussed. Methods such as cDNA microarray anal. and phage display screening were used to identify relevant markers and use these for selective drug targeting to the lymphatic vessel. The current targeting technologies made it possible to develop almost any drug into a targeted compound, thus increasing the potency of the drug at the intended target tissue, while reducing any side effects in the body.

L32 ANSWER 81 OF 121 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN
DUPLICATE 43

2001:592326 The Genuine Article (R) Number: 453TH. Molecular regulation of lymphangiogenesis and targets for tissue oedema. Karkkainen M J (Reprint); Jussila L; Ferrell R E; Finegold D N; **Alitalo K.** Univ Helsinki, Mol Canc Biol Lab, POB 21 Haartmaninkatu 3, FIN-00014 Helsinki, Finland (Reprint); Univ Helsinki, Mol Canc Biol Lab, FIN-00014 Helsinki, Finland; Univ Helsinki, Ludwig Inst Canc Res, FIN-00014 Helsinki, Finland; Univ Pittsburgh, Grad Sch Publ Hlth, Dept Human Genet, Pittsburgh, PA 15261 USA; Univ Pittsburgh, Dept Pediat, Pittsburgh, PA 15213 USA. TRENDS IN MOLECULAR MEDICINE (JAN 2001) Vol. 7, No. 1, pp. 18-22. ISSN: 1471-4914. Publisher: ELSEVIER SCI LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, OXON, ENGLAND. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB New insight has recently been obtained into the molecular mechanisms regulating the function of lymphatic endothelial cells. Vascular endothelial growth factors-C and -D have been shown to stimulate lymphangiogenesis. and their receptor **VEGFR-3** has been linked to human hereditary lymphoedema. although there is evidence that other genes are also involved. These data suggest that it may become possible to stimulate lymphatic growth and function and to treat tissue oedema involved in many diseases.

L32 ANSWER 82 OF 121 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
DUPLICATE 44

2001:230941 Document No.: PREV200100230941. Vascular endothelial growth factor C (**VEGF-C**) DELTACys156 protein and gene, and uses thereof. **Alitalo, Kari** [Inventor, Reprint author]; Joukov, Vladimir [Inventor]. Espoo, Finland. ASSIGNEE: Helsinki University Licensing, Ltd., Helsinki, Finland. Patent Info.: US 6130071 20001010. Official Gazette of the United States Patent and Trademark Office Patents, (Oct. 10, 2000) Vol. 1239, No. 2. e-file. CODEN: OGUPE7. ISSN: 0098-1133. Language: English.

AB Provided are purified and isolated **VEGF-C** cysteine deletion variants that bind to Flt4 receptor tyrosine kinase (**VEGFR-3**) but demonstrate reduced binding (relative to **VEGF-C**) to kdr receptor tyrosine kinase (**VEGFR-2**); polynucleotides encoding the polypeptide; vectors and host cells that embody the polynucleotides; pharmaceutical compositions and diagnostic reagents comprising the polypeptides; and methods of making and using the foregoing.

L32 ANSWER 83 OF 121 CAPLUS COPYRIGHT 2006 ACS on STN

2000:707330 Document No. 133:280149 Screening and therapy for lymphatic disorders involving the FLT4 receptor tyrosine kinase (**VEGFR-3**). Ferrell, Robert E.; **Alitalo, Kari**; Finegold, David N.; Karkkainen, Marika (Ludwig Institute for Cancer Research, USA; Helsinki University Licensing Ltd. Oy; University of Pittsburgh of the Commonwealth System of Higher Education). PCT Int. Appl. WO 2000058511 A1 20001005, 76 pp. DESIGNATED STATES: W: CA, JP, US. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US6133 19990326.

AB The present invention provides materials and methods for screening for and treating hereditary lymphedema in human subjects. The invention is based on the discovery that the lymphedema phenotype correlates with a missense mutation in the **VEGFR-3** gene, which maps to chromosome 5q34-q35 and encodes FLT4 receptor tyrosine kinase. **VEGFR-3** acts as a high affinity receptor for vascular endothelial growth factor C (**VEGF-C**), a growth factor whose effects include modulation of the growth of the lymphatic vascular network.

L32 ANSWER 84 OF 121 CAPLUS COPYRIGHT 2006 ACS on STN

2000:260057 Document No. 132:298824 Flt4 (**VEGFR-3**) as a target for tumor imaging and anti-tumor therapy. **Alitalo, Kari**; Kaipainen, Arja; Valltola, Reija; Jussila, Lotta (Ludwig Institute for Cancer Research, USA; Helsinki University Licensing Ltd. Oy). PCT Int. Appl. WO 2000021560 A1 20000420, 148 pp. DESIGNATED STATES: W: AU, CA, CN, JP, NO, NZ; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US23525 19991008. PRIORITY: US 1998-169079 19981009.

AB The present invention provides purified Flt4 receptor tyrosine kinase polypeptides and fragments thereof, polynucleotides encoding such polypeptides, antibodies that specifically bind such polypeptides, and uses therefor.

L32 ANSWER 85 OF 121 MEDLINE on STN DUPLICATE 45

2001096205. PubMed ID: 11090062. **VEGF-C** signaling pathways through **VEGFR-2** and **VEGFR-3** in vasculoangiogenesis and hematopoiesis. Hamada K; Oike Y; Takakura N; Ito Y; Jussila L; Dumont D J; **Alitalo K**; Suda T. (Department of Cell Differentiation, Institute of Molecular Embryology and Genetics, Kumamoto University, Japan.) Blood, (2000 Dec 1) Vol. 96, No. 12, pp. 3793-800. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB Signaling by vascular endothelial growth factors (VEGFs) through VEGF receptors (VEGFRs) plays important roles in vascular development and hematopoiesis. The authors analyzed the function of **VEGF-C** signaling through both **VEGFR-2** and **VEGFR-3** in vasculoangiogenesis and hematopoiesis using a coculture of para-aortic splanchnopleural mesoderm (P-Sp) explants from mouse embryos with stromal cells (OP9). Vasculogenesis and angiogenesis were evaluated by the extent of vascular bed and network formation, respectively. Addition of **VEGF-C** to the P-Sp culture enhanced vascular bed formation and suppressed definitive hematopoiesis. Both vascular bed and network formations were completely suppressed by addition of soluble VEGFR-1-Fc competitor protein. Formation of vascular beds but not networks could be rescued by **VEGF-C** in the presence of

the competitor, while both were rescued by VEGF-A. **VEGFR-3**-deficient embryos show the abnormal vasculature and severe anemia. Consistent with these in vivo findings, vascular bed formation in the P-Sp from the **VEGFR-3**-deficient embryos was enhanced to that in wild-type or heterozygous embryos, and hematopoiesis was severely suppressed. When **VEGFR-3**-Fc chimeric protein was added to trap endogenous **VEGF-C** in the P-Sp culture of the **VEGFR-3**-deficient embryos, vascular bed formation was suppressed and hematopoiesis was partially rescued. These results demonstrate that because **VEGF-C** signaling through VEGFR-2 works synergistically with VEGF-A, the binding of **VEGF-C** to **VEGFR-3** consequently regulates VEGFR-2 signaling. In **VEGFR-3**-deficient embryos, an excess of **VEGF-C** signals through VEGFR-2 induced the disturbance of vasculogenesis and hematopoiesis during embryogenesis. This indicates that elaborated control through **VEGFR-3** signaling is critical in vasculoangiogenesis and hematopoiesis. (Blood. 2000;96:3793-3800)

L32 ANSWER 86 OF 121 MEDLINE on STN DUPLICATE 46

2001021068. PubMed ID: 11023993. **VEGF-C** and VEGF-D expression in neuroendocrine cells and their receptor, **VEGFR-3**, in fenestrated blood vessels in human tissues. Partanen T A; Arola J; Saaristo A; Jussila L; Ora A; Miettinen M; Stacker S A; Achen M G; **Alitalo K**. (Molecular/Cancer Biology Laboratory and Department of Pathology, Haartman Institute, University of Helsinki, 00014 Helsinki, Finland.) The FASEB journal : official publication of the Federation of American Societies for Experimental Biology, (2000 Oct) Vol. 14, No. 13, pp. 2087-96. Journal code: 8804484. ISSN: 0892-6638. Pub. country: United States. Language: English.

AB Recently, vascular endothelial growth factor receptor 3 (**VEGFR-3**) has been shown to provide a specific marker for lymphatic endothelia in certain human tissues. In this study, we have investigated the expression of **VEGFR-3** and its ligands **VEGF-C** and VEGF-D in fetal and adult tissues. **VEGFR-3** was consistently detected in the endothelium of lymphatic vessels such as the thoracic duct, but fenestrated capillaries of several organs including the bone marrow, splenic and hepatic sinusoids, kidney glomeruli and endocrine glands also expressed this receptor. **VEGF-C** and VEGF-D, which bind both VEGFR-2 and **VEGFR-3** were expressed in vascular smooth muscle cells. In addition, intense cytoplasmic staining for **VEGF-C** was observed in neuroendocrine cells such as the alpha cells of the islets of Langerhans, prolactin secreting cells of the anterior pituitary, adrenal medullary cells, and dispersed neuroendocrine cells of the gastrointestinal tract. VEGF-D was observed in the innermost zone of the adrenal cortex and in certain dispersed neuroendocrine cells. These results suggest that **VEGF-C** and VEGF-D have a paracrine function and perhaps a role in peptide release from secretory granules of certain neuroendocrine cells to surrounding capillaries.

L32 ANSWER 87 OF 121 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

2000:356704 The Genuine Article (R) Number: 312XY. Vascular endothelial growth factor receptor-3 in lymphangiogenesis in wound healing. Paavonen K; Puolakkainen P; Jussila L; Jahkola T; **Alitalo K (Reprint)**. Univ Helsinki, Haartman Inst, Mol Canc Biol Lab, POB 21, Haartmaninkatu 3, Helsinki 00014, Finland (Reprint); Univ Helsinki, Haartman Inst, Mol Canc Biol Lab, Helsinki 00014, Finland; Univ Helsinki, Cent Hosp, Dept Surg, Helsinki, Finland; Univ Helsinki, Cent Hosp, Dept Plast Surg, Helsinki, Finland. AMERICAN JOURNAL OF PATHOLOGY (MAY 2000) Vol. 156, No. 5, pp. 1499-1504. ISSN: 0002-9440. Publisher: AMER SOC INVESTIGATIVE PATHOLOGY, INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3993 USA.

Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Vascular endothelial growth factor receptor-3 (**VEGFR-3**) is essential for embryonic cardiovascular development, but thereafter becomes confined to the lymphatic endothelium in adult tissues. We have here studied **VEGFR-3** expression in experimental wounds of pigs and chronic inflammatory wounds of humans. In healing incisional and punch biopsy wounds made in the dorsal skin of pigs, angiogenic blood vessels, identified by use of the blood vascular endothelial markers vWF: and PAL-E and the basal lamina protein laminin, developed into the granulation tissue stroma from day 4 onward, being most abundant on days 5 and 6 and regressing thereafter. **VEGFR-3**-positive vessels were observed in the granulation tissue from day 5 onward. These vessels were distinct from the PAL-E/laminin/vWF-positive vessels and fewer in number, and they appeared to sprout from pre-existing **VEGFR-3**-positive lymphatic vessels at the wound edge. Unlike the blood vessels, very few **VEGFR-3**-positive lymphatic vessels persisted on day 9 and none on day 14. In chronic wounds such as ulcers and decubitus wounds of the lower extremity of humans, **VEGFR-3** was also weakly expressed in the vascular endothelium. Our results suggest that transient lymphangiogenesis occurs in parallel With angiogenesis in healing wounds and that **VEGFR-3** becomes up-regulated in blood vessel endothelium in chronic inflammatory wounds.

L32 ANSWER 88 OF 121 MEDLINE on STN DUPLICATE 47
2000391496. PubMed ID: 10887117. Involvement of vascular endothelial growth factor receptor-3 in maintenance of integrity of endothelial cell lining during tumor angiogenesis. **Kubo H**; Fujiwara T; Jussila L; Hashi H; Ogawa M; Shimizu K; Awane M; Sakai Y; Takabayashi A; **Alitalo K**; Yamaoka Y; Nishikawa S I. (Departments of Gastroenterological Surgery and Molecular Genetics, Graduate School of Medicine, Kyoto University, Kyoto, Japan.. kubofit@kuhp.kyoto-u.ac.jp) . Blood, (2000 Jul 15) Vol. 96, No. 2, pp. 546-53. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB Vascular endothelial growth factor (VEGF) plays a major role in tumor angiogenesis. **VEGF-C**, however, is thought to stimulate the growth of lymphatic vessels because an expression of its specific receptor, VEGF receptor-3 (**VEGFR-3**), was demonstrated to be restricted to lymphatic vessels. Here we demonstrate that the inactivation of **VEGFR-3** by a novel blocking monoclonal antibody (mAb) suppresses tumor growth by inhibiting the neo-angiogenesis of tumor-bearing tissues. Although **VEGFR-3** is not expressed in adult blood vessels, it is induced in vascular endothelial cells of the tumor-bearing tissues. Hence, **VEGFR-3** is another receptor tyrosine kinase involved in tumor-induced angiogenesis. Micro-hemorrhage in the tumor-bearing tissue was the most conspicuous histologic finding specific to AFL4 mAb-treated mice. Scanning microscopy demonstrated disruptions of the endothelial lining of the postcapillary venule, probably the cause of micro-hemorrhage and the subsequent collapse of the proximal vessels. These findings suggest the involvement of **VEGFR-3** in maintaining the integrity of the endothelial lining during angiogenesis. Moreover, our results suggest that the **VEGF-C/VEGFR-3** pathway may serve another candidate target for cancer therapy. (Blood. 2000;96:546-553)

L32 ANSWER 89 OF 121 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
2001:312511 Document No.: PREV200100312511. **VEGF-C** signaling through Flt-4 (**VEGFR3**) mediates leukemic cell proliferation and survival. Choy, M. [Reprint author]; Dias, S. [Reprint author]; Alitalo, R.; **Alitalo, K.**; Rafii, S. [Reprint author]. Cornell U. Med.

College, New York, NY, USA. Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 502a-503a. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology. San Francisco, California, USA. December 01-05, 2000. American Society of Hematology.

CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

AB Recent findings have documented the relationship between leukemia and angiogenesis. In leukemia, increased bone marrow vessel density and vascular endothelial growth factor (VEGF) plasma levels correlate with poor prognosis. Leukemic cells secrete endothelial growth factors such as VEGF to enhance endothelial cell (EC) survival and proliferation while in turn ECs release growth factors such as GM-CSF to support leukemic cell growth. Given that other VEGF family members may play a role in leukemia biology, we speculated that **VEGF-C** may also modulate leukemic cell growth. **VEGF-C**, which binds VEGFR-2 (KDR) and **VEGFR-3** (Flt-4), was recently shown to be elaborated by subsets of leukemic cells. Similar to VEGF, **VEGF-C** increases EC migration and proliferation. However, in contrast to VEGF, it is expressed by various ECs including lymphatic EC and primary human umbilical vein EC (HUVEC) as well as certain solid and liquid tumors. Since the **VEGF-C** specific receptor, Flt-4, is expressed on primary leukemia cells and cell lines, we hypothesized that it may play a role in leukemia cell growth and survival. In this study, the leukemia cell lines THP-1 and HEL were found to express functional Flt-4 receptors that phosphorylate upon stimulation by either **VEGF-C** or mutant **VEGF-C** (which only signals through Flt-4, but not KDR). Treatment with **VEGF-C** or its mutant in serum free conditions increased THP-1 and HEL proliferation by 20-30% over a 24-48 hr period. Additionally, both **VEGF-C** and its mutant enhanced THP-1 and HEL survival by 30-40%, as determined by Trypan blue exclusion and Annexin V staining. Its pro-survival effects were further demonstrated by an upregulation of the anti-apoptotic protein Bcl-2 in HEL and THP-1 cells following 24 hour serum-free treatment with either **VEGF-C** or its mutant. These results suggest that **VEGF-C** exerts both mitogenic and pro-survival effects on leukemic cells through its receptor Flt-4. Given that ECs as well as leukemic cells secrete **VEGF-C**, its production may support leukemic cell proliferation and survival through a Flt-4 mediated autocrine and/or paracrine mechanism. In this context, we demonstrate that leukemic cells produce pro-inflammatory cytokines such as IL-1 and TNF which increases **VEGF-C** production by HUVEC, generating a paracrine loop to support leukemia growth and survival. In turn, enhanced leukemia cell survival and proliferation may increase blood vessel density by elevating levels of leukemia-derived proangiogenic factors such as VEGF and FGF-2. These results identify the **VEGF-C**/Flt-4 pathway as a potential target for therapeutic intervention in subsets of human acute leukemias.

L32 ANSWER 90 OF 121 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

2001:100428 Document No.: PREV200100100428. VEGFs and receptors regulating angiogenesis and lymphangiogenesis. **Alitalo, K. K.** [Reprint author]. Molecular/Cancer Biology, Haartman Institute, Helsinki, Finland. Journal of Submicroscopic Cytology and Pathology, (July, 2000) Vol. 32, No. 3, pp. 386. print.
Meeting Info.: XIth International Vascular Biology Meeting. Geneva, Switzerland. September 05-09, 2000.
ISSN: 1122-9497. Language: English.

L32 ANSWER 91 OF 121 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

2000:464048 Document No.: PREV200000464048. An avian model for studies of

embryonic lymphangiogenesis. Wilting, J. [Reprint author]; Schneider, N.; Papoutsi, M.; **Alitalo, K.**; Christ, B.. Anatomisches Institut der Albert-Ludwigs-Universitaet Freiburg, Albertstrasse 17, D-79104, Freiburg im Breisgau, Germany. Lymphology, (September, 2000) Vol. 33, No. 3, pp. 81-94. print.

CODEN: LYMPBN. ISSN: 0024-7766. Language: English.

- AB Embryonic development of lymphatics (lymphangiogenesis) in recent years has rarely been studied experimentally. Using an avian model, we showed that both intra- and extra-embryonic blood vessels of chick and quail embryos are accompanied by lymphatics. The lymphatics of the chorioallantoic membrane (CAM) are drained by lymphatic trunks of the umbilicus and are connected to the posterior lymph hearts. Intra-embryonic lymphatics are drained via paired thoracic ducts into the jugulo-subclavian junction. The lymphatic endothelial cells are characterized by the expression of Vascular Endothelial Growth Factor Receptors (VEGFR) -2 and -3. Application of **VEGF-C**, the ligand of these two receptors, on the differentiated CAM, induces proliferation of lymphatic endothelial cells and formation of huge lymphatic sinuses. These lymphatics derive from pre-existing lymphatic endothelial cells, whereas, in early embryos lymphangioblasts are present in the mesenchyme. This phenomenon can be demonstrated by interspecific grafting experiments between chick and quail embryos. Together with the early lymph sacs, the lymphangioblasts form the embryonic lymphatic system. Our studies demonstrate the importance of lymphangioblasts and lymphangiogenic growth factors in embryonic lymphangiogenesis.

L32 ANSWER 92 OF 121 CAPLUS COPYRIGHT 2006 ACS on STN

2000:773478 Document No. 134:66223 Growth factors regulating lymphatic vessels. Lymboussaki, A.; Achen, M. G.; Stacker, S. A.; **Alitalo, K.** (Molecular/Cancer Biology Laboratory, Haartman Institute, University of Helsinki, Finland, 00014, Finland). Current Topics in Microbiology and Immunology, 251(Lymphoid Organogenesis), 75-82 (English) 2000. CODEN: CTMIA3. ISSN: 0070-217X. Publisher: Springer-Verlag.

- AB A review with 44 refs. Over the past 10 yr, much has been learned about the mol. control of angiogenesis, but only recently have the first regulators of lymphangiogenesis been identified. The availability of **VEGF-C** and VEGF-D offers the opportunity to induce lymphangiogenesis in the clinic, which may be useful for treatment of lymphedema. The expression of **VEGF-C** and VEGF-D in tumors raises the possibility of tumor lymphangiogenesis. Despite involvement of the lymphatics in tumor metastasis, little is known about the relationship between tumor cells and the lymphatic endothelium. The route by which a tumor metastasizes may, in part, be determined by the angiogenic/lymphangiogenic growth factors secreted by tumor cells that modulate the prevalence of vessels in a tumor. Specific inhibitors of **VEGF-C**, VEGF-D or **VEGFR-3** will be required to address this important issue.

L32 ANSWER 93 OF 121 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
DUPLICATE 48

2001:299450 Document No.: PREV200100299450. **VEGF-C** signaling pathways through VEGFR-2 and **VEGFR-3** in vasculo-angiogenesis and hematopoiesis. Hamada, Koichi [Reprint author]; Oike, Yuichi [Reprint author]; Takakura, Nobuyuki [Reprint author]; Ito, Yasuhiro [Reprint author]; Jussila, Lotta; Dumont, Daniel J.; **Alitalo, Kari**; Suda, Toshio [Reprint author]. Cell Differentiation, Kumamoto University, Kumamoto, Japan. Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 35a. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology. San Francisco, California, USA. December 01-05, 2000. American Society of Hematology.

CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

- AB Embryonic development of blood vessels from endothelial cells consists of

vasculogenesis and angiogenesis. Vasculo-angiogenesis is regulated by paracrine signals, many of which are protein ligands that bind to and modulate the activity of transmembrane receptor tyrosine kinases (RTKs). Among the vasculo-angiogenic factors, vascular endothelial growth factor (VEGF) family members and their receptors are known to be critical for vasculo-angiogenesis and hematopoiesis. To clarify these functional differences in **VEGF-C** signaling through VEGFR-2 and **VEGFR-3**, we analyzed using a coculture of para-aortic splanchnopleural mesoderm (P-Sp) explants from mouse embryos with stromal cells (OP9). This culture system is used to assay both vasculo-angiogenesis and hematopoiesis (Immunity 9:677-86, 1998). Vasculogenesis and angiogenesis were evaluated by the extent of vascular bed and network formation, respectively. Addition of **VEGF-C** to the P-Sp culture enhanced vascular bed formation and suppressed definitive hematopoiesis. Both vascular bed and network formations were completely suppressed by addition of soluble VEGFR-1-Fc competitor protein. Formation of vascular beds but not networks could be rescued by **VEGF-C** in the presence of the competitor, while both were rescued by VEGF-A. **VEGFR-3** deficient embryos show the abnormal vasculature and severe anemia. Consistent with these in vivo findings, vascular bed formation in the P-Sp from the **VEGFR-3** deficient embryos was enhanced to that in wild-type or heterozygous embryos and hematopoiesis was severely suppressed. When **VEGFR-3**-Fc chimeric protein was added to trap endogenous **VEGF-C** in the P-Sp culture of the **VEGFR-3** deficient embryos, vascular bed formation was suppressed and hematopoiesis was partially rescued. These results demonstrate that since **VEGF-C** signaling through VEGFR-2 works synergistically with VEGF-A, the binding of **VEGF-C** to **VEGFR-3** consequently regulates VEGFR-2 signaling. In **VEGFR-3** deficient embryos, an excess of **VEGF-C** signals through VEGFR-2 induced the disturbance of vasculogenesis and hematopoiesis during embryogenesis. This indicates that elaborated control through **VEGFR-3** signaling is critical in vasculo-angiogenesis and hematopoiesis.

L32 ANSWER 94 OF 121 MEDLINE on STN DUPLICATE 49
 2000390246. PubMed ID: 10880369. Vascular endothelial growth factor-C and its receptor **VEGFR-3** in the nasal mucosa and in nasopharyngeal tumors. Saaristo A; Partanen T A; Arola J; Jussila L; Hytonen M; Makitie A; Vento S; Kaipainen A; Malmberg H; **Alitalo K**. (Molecular/Cancer Biology Laboratory and Ludwig Institute for Cancer Research, University of Helsinki, Finland.) The American journal of pathology, (2000 Jul) Vol. 157, No. 1, pp. 7-14. Journal code: 0370502. ISSN: 0002-9440. Pub. country: United States. Language: English.

AB Vascular endothelial growth factors (VEGFs) and their receptors (VEGFRs) are important regulators of blood and lymphatic vessel growth and vascular permeability. Both blood and lymphatic vessels of the upper respiratory tract play important roles in pathological conditions, such as infections and tumors. Here we have studied the expression of **VEGF-C** and its receptor **VEGFR-3** in the upper respiratory system by Northern blot analysis and immunohistochemistry of human tissues, and in situ mRNA hybridization of developing mouse embryos and beta-galactosidase staining of mouse embryos having a LacZ marker gene in the **VEGFR-3** gene locus. The results demonstrate expression of **VEGF-C** and **VEGFR-3** in the developing and adult nasal respiratory epithelium and in the nasal vascular plexus, respectively. Unlike in most other tissues, in the nasal mucosa **VEGFR-3** is expressed in both blood and lymphatic vessels. Expression of **VEGF-C** was also detected in nasal and nasopharyngeal tumor islands, which were surrounded by **VEGFR-3**-positive angiogenic blood vessels. These results suggest that **VEGF-C** and **VEGFR-**

3 have a role in the development of the nasal submucosal vascular plexus and in its normal function and that they are associated with angiogenesis in nasal and nasopharyngeal tumors.

L32 ANSWER 95 OF 121 MEDLINE on STN DUPLICATE 50
2000011413. PubMed ID: 10542248. Biosynthesis of vascular endothelial growth factor-D involves proteolytic processing which generates non-covalent homodimers. Stacker S A; Stenvers K; Caesar C; Vitali A; Domagala T; Nice E; Roufail S; Simpson R J; Moritz R; Karpanen T; **Alitalo K**; Achen M G. (Ludwig Institute for Cancer Research, Royal Melbourne Hospital, Parkville, Victoria 3050, Australia.. steven.stacker@ludwig.edu.au) . The Journal of biological chemistry, (1999 Nov 5) Vol. 274, No. 45, pp. 32127-36. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Vascular endothelial growth factor-D (VEGF-D) binds and activates the endothelial cell tyrosine kinase receptors VEGF receptor-2 (VEGFR-2) and VEGF receptor-3 (**VEGFR-3**), is mitogenic for endothelial cells, and shares structural homology and receptor specificity with **VEGF-C**. The primary translation product of VEGF-D has long N- and C-terminal polypeptide extensions in addition to a central VEGF homology domain (VHD). The VHD of VEGF-D is sufficient to bind and activate VEGFR-2 and **VEGFR-3**. Here we report that VEGF-D is proteolytically processed to release the VHD. Studies in 293EBNA cells demonstrated that VEGF-D undergoes N- and C-terminal cleavage events to produce numerous secreted polypeptides including a fully processed form of M(r) approximately 21,000 consisting only of the VHD, which is predominantly a non-covalent dimer. Biosensor analysis demonstrated that the VHD has approximately 290- and approximately 40-fold greater affinity for VEGFR-2 and **VEGFR-3**, respectively, compared with unprocessed VEGF-D. In situ hybridization demonstrated that embryonic lung is a major site of expression of the VEGF-D gene. Processed forms of VEGF-D were detected in embryonic lung indicating that VEGF-D is proteolytically processed in vivo.

L32 ANSWER 96 OF 121 MEDLINE on STN DUPLICATE 51
1999419043. PubMed ID: 10488101. Vascular endothelial growth factor-C stimulates the migration and proliferation of Kaposi's sarcoma cells. Marchio S; Primo L; Pagano M; Palestro G; Albin A; Veikkola T; Cascone I; **Alitalo K**; Bussolino F. (Institute for Cancer Research and Treatment, Department of Genetics, University of Torino Medical School, 10060 Candiolo, Italy.) The Journal of biological chemistry, (1999 Sep 24) Vol. 274, No. 39, pp. 27617-22. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Recent evidence suggesting vascular endothelial growth factor-C (**VEGF-C**), which is a regulator of lymphatic and vascular endothelial development, raised the question whether this molecule could be involved in Kaposi's sarcoma (KS), a strongly angiogenic and inflammatory tumor often associated with infection by human immunodeficiency virus-1. This disease is characterized by the presence of a core constituted of three main populations of "spindle" cells, having the features of lymphatic/vascular endothelial cells, macrophagic/dendritic cells, and of a mixed macrophage-endothelial phenotype. In this study we evaluated the biological response of KS cells to **VEGF-C**, using an immortal cell line derived from a KS lesion (KS IMM), which retains most features of the parental tumor and can induce KS-like sarcomas when injected subcutaneously in nude mice. We show that **VEGFR-3**, the specific receptor for **VEGF-C**, is expressed by KS IMM cells grown in vitro and in vivo. In vitro, **VEGF-C** induces the tyrosine phosphorylation of VEGFR-2, a receptor also for VEGF-A, as well as that of **VEGFR-3**. The activation of these two receptors in KS IMM cells is followed by a dose-responsive mitogenic and motogenic response. The stimulation of KS IMM cells with a mutant **VEGF-**

C unable to bind and activate VEGFR-2 resulted in no proliferative response and in a weak motogenic stimulation, suggesting that VEGFR-2 is essential in transducing a proliferative signal and cooperates with **VEGFR-3** in inducing cell migration. Our data add new insights on the pathogenesis of KS, suggesting that the involvement of endothelial growth factors may not only determine KS-associated angiogenesis, but also play a critical role in controlling KS cell growth and/or migration and invasion.

- L32 ANSWER 97 OF 121 MEDLINE on STN DUPLICATE 52
 1999263374. PubMed ID: 10329591. **VEGFR-3** and its ligand **VEGF-C** are associated with angiogenesis in breast cancer. Valtola R; Salven P; Heikkila P; Taipale J; Joensuu H; Rehn M; Pihlajaniemi T; Weich H; deWaal R; **Alitalo K.** (Molecular/Cancer Biology Laboratory, Department of Pathology, Haartman Institute, University of Helsinki, Finland.) The American journal of pathology, (1999 May) Vol. 154, No. 5, pp. 1381-90. Journal code: 0370502. ISSN: 0002-9440. Pub. country: United States. Language: English.
- AB Recently, monoclonal antibodies against the human vascular endothelial growth factor receptor **VEGFR-3** were shown to provide a specific antigenic marker for lymphatic endothelium in various normal tissues. In this study we have investigated the expression of **VEGFR-3** and its ligand **VEGF-C** in normal breast tissue and in breast tumors by immunohistochemistry. **VEGFR-3** was weakly expressed in capillaries of normal breast tissue and in fibroadenomas. In intraductal breast carcinomas, **VEGFR-3** was prominent in the "necklace" vessels adjacent to the basal lamina of the tumor-filled ducts. VEGF receptor 1 and 2 as well as blood vessel endothelial and basal lamina markers were colocalized with **VEGFR-3** in many of these vessels. Antibodies against smooth muscle alpha-actin gave a weak staining of the necklace vessels, suggesting that they were incompletely covered by pericytes/smooth muscle cells. A highly elevated number of **VEGFR-3** positive vessels was found in invasive breast cancer in comparison with histologically normal breast tissue (P < 0.0001, the Mann-Whitney test). **VEGF-C** was located in the cytoplasm of intraductal and invasive cancer cells. The results demonstrate that the expression of **VEGFR-3** becomes up-regulated in the endothelium of angiogenic blood vessels in breast cancer. The results also suggest that **VEGF-C** secreted by the intraductal carcinoma cells acts predominantly as an angiogenic growth factor for blood vessels, although this paracrine signaling network between the cancer cells and the endothelium may also be involved in modifying the permeabilities of both blood and lymphatic vessels and metastasis formation.

- L32 ANSWER 98 OF 121 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN DUPLICATE 53
 1999:267298 Document No.: PREV199900267298. Vascular endothelial growth factor-C (**VEGF-C**) and its receptors VEGFR-2 and **VEGFR-3** are expressed in AIDS-associated Kaposi's sarcoma. Skobe, M. [Reprint author]; Brown, L.; Tognazzi, K.; Groupman, J.; **Alitalo, K.**; Detmar, M. [Reprint author]. Cutaneous Biology Research Center, Massachusetts General Hospital, Charlestown, MA, USA. Journal of Investigative Dermatology, (April, 1999) Vol. 112, No. 4, pp. 618. print.
 Meeting Info.: 60th Annual Meeting of the Society for Investigative Dermatology. Chicago, Illinois, USA. May 5-9, 1999.
 CODEN: JIDEAE. ISSN: 0022-202X. Language: English.

- L32 ANSWER 99 OF 121 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
 1999:175777 Document No.: PREV199900175777. Upregulation of the **VEGF**

-C/**VEGFR-3** signalling pathway in breast cancer angiogenesis. Valtola, R.; Salven, P.; Heikkila, P.; Taipale, J.; Joensuu, H.; Rehr, M.; Pihlajaniemi, T.; Weich, H.; Dewall, R.; **Alitalo, K.** Molecular/Cancer Biol. Lab., Haartman Inst., P.O. Box 21, Univ. Helsinki, 00014 Helsinki, Finland. Proceedings of the American Association for Cancer Research Annual Meeting, (March, 1999) Vol. 40, pp. 369. print.
Meeting Info.: 90th Annual Meeting of the American Association for Cancer Research. Philadelphia, Pennsylvania, USA. April 10-14, 1999. American Association for Cancer Research.
ISSN: 0197-016X. Language: English.

- L32 ANSWER 100 OF 121 MEDLINE on STN DUPLICATE 54
1999316901. PubMed ID: 10390013. Vascular endothelial growth factor-C expression in human prostatic carcinoma and its relationship to lymph node metastasis. Tsurusaki T; Kanda S; Sakai H; Kanetake H; Saito Y; **Alitalo K**; Koji T. (Department of Urology, Nagasaki University School of Medicine, Japan.) British journal of cancer, (1999 Apr). Vol. 80, No. 1-2, pp. 309-13. Journal code: 0370635. ISSN: 0007-0920. Pub. country: SCOTLAND: United Kingdom. Language: English.
- AB Lymph node dissemination is a major prognostic factor in human cancer. However, the molecular mechanisms underlying lymph node metastasis are poorly understood. Recently, vascular endothelial growth factor-C (**VEGF-C**) was identified as a ligand for VEGF receptor-3 (**VEGFR-3**/Flt-4) and the expression of **VEGFR-3** was found to be highly restricted to the lymphatic endothelial cells. In this report, we investigated the expression of **VEGF-C** and **VEGFR-3** in human prostatic carcinoma tissue by using in situ hybridization and immunohistochemical staining respectively. Expression of **VEGF-C** mRNA in prostatic carcinoma was significantly higher in lymph node-positive group than in lymph node-negative group. In addition, the number of **VEGFR-3**-positive vessels was increased in stroma surrounding **VEGF-C**-positive prostatic carcinoma cells. These results suggest that the expression of **VEGF-C** in prostatic carcinoma cells is implicated in the lymph node metastasis.
- L32 ANSWER 101 OF 121 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN
1999:165869 The Genuine Article (R) Number: 171DU. Expression of vascular endothelial growth factor receptor-3 and podoplanin suggests a lymphatic endothelial cell origin of Kaposi's sarcoma tumor cells. Weninger W; Partanen T A; Breiteneder-Geleff S; Mayer C; Kowalski H; Mildner M; Pammer J; Sturzl M; Kerjaschki D; **Alitalo K**; Tschachler E (Reprint). Univ Vienna, Sch Med, Dept Dermatol, Inst Clin Pathol, Div Immunol Allergy & Infect Dis, Wahringer Gurtel 18-20, A-1090 Vienna, Austria (Reprint); Univ Vienna, Sch Med, Dept Dermatol, Inst Clin Pathol, Div Immunol Allergy & Infect Dis, A-1090 Vienna, Austria; Univ Helsinki, Haartman Inst, Dept Pathol, Mol Canc Biol Lab, FIN-00014 Helsinki, Finland; GSF Forschungszentrum Umwelt & Gesundheit GMBH, Inst Mol Virol, Nueherberg, Germany. LABORATORY INVESTIGATION (FEB 1999) Vol. 79, No. 2, pp. 243-251. ISSN: 0023-6837. Publisher: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
- AB Despite intensive research over the past decade, the exact lineage relationship of Kaposi's sarcoma (KS) tumor cells has not yet been settled. In the present study, we investigated the expression of two markers for lymphatic endothelial cells (EC), ie, vascular endothelial growth factor receptor-3 (**VEGFR-3**) and podoplanin, in AIDS and classic KS. Both markers were strongly expressed by cells lining irregular vascular spaces in early KS lesions and by tumor cells in advanced KS. Double-staining experiments by confocal laser microscopy established that **VEGFR-3**-positive and

podoplanin-positive cell populations were identical and uniformly expressed CD31. By contrast, these cells were negative for CD45, CD68, and PAL-E, excluding their hemopoietic and blood vessel endothelial cell nature. Podoplanin expression in primary KS tumor lysates was confirmed by Western blot analysis. Both splice variants of **VEGFR-3** were found in KS-tumor-derived RNA by RT-PCR. In contrast to KS tumor cells in situ, no expression of **VEGFR-3** and podoplanin was detected in any of four KS-derived spindle cell cultures and in one KS-derived autonomously growing cell line (KS Y-1). Our findings that KS tumor cells express two lymphatic EC markers in situ strongly suggest that they are related to or even derived from the lymphatic EC lineage. Lack of these antigens on cultured cells derived from KS lesions indicates that they might not represent tumor cells that grow in tissue culture, but rather other cell types present in KS lesions.

L32 ANSWER 102 OF 121 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on
STN DUPLICATE 55

1999:167187 Document No.: PREV199900167187. **VEGF-C** and **VEGFR-3** expression and biosynthesis in the lymphatic endothelium. Leak, L. V. [Reprint author]; **Alitalo, K.**; Wood, C.. Coll. Med., Howard Univ., Washington, DC 20059, USA. FASEB Journal, (March 12, 1999) Vol. 13, No. 4 PART 1, pp. A32. print. Meeting Info.: Annual Meeting of the Professional Research Scientists for Experimental Biology 99. Washington, D.C., USA. April 17-21, 1999. CODEN: FAJOEC. ISSN: 0892-6638. Language: English.

L32 ANSWER 103 OF 121 MEDLINE on STN DUPLICATE 56

1999045694. PubMed ID: 9826710. Vascular endothelial growth factor C induces angiogenesis in vivo. Cao Y; Linden P; Farnebo J; Cao R; Eriksson A; Kumar V; Qi J H; Claesson-Welsh L; **Alitalo K.** (Laboratory of Angiogenesis Research, Microbiology and Tumor Biology Center, Karolinska Institutet, S-171 77 Stockholm, Sweden.. yihai.cao@mtc.ki.se) . Proceedings of the National Academy of Sciences of the United States of America, (1998 Nov 24) Vol. 95, No. 24, pp. 14389-94. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Vascular endothelial growth factor C (**VEGF-C**) recently has been described to be a relatively specific growth factor for the lymphatic vascular system. Here we report that ectopic application of recombinant **VEGF-C** also has potent angiogenic effects in vivo. **VEGF-C** is sufficiently potent to stimulate neovascularization from limbal vessels in the mouse cornea. Similar to VEGF, the angiogenic response of corneas induced by **VEGF-C** is intensive, with a high density of new capillaries. However, the outgrowth of microvessels stimulated by **VEGF-C** was significantly longer than that induced by VEGF. In the developing embryo, **VEGF-C** was able to induce branch sprouts from the established blood vessels. **VEGF-C** also induced an elongated, spindle-like cell shape change and actin reorganization in both VEGF receptor (VEGFR)-2 and **VEGFR-3**-overexpressing endothelial cells, but not in VEGFR-1-expressing cells. Further, both VEGFR-2 and **VEGFR-3** could mediate proliferative and chemotactic responses in endothelial cells on **VEGF-C** stimulation. Thus, **VEGF-C** may regulate physiological angiogenesis and participate in the development and progression of angiogenic diseases in addition to lymphangiogenesis.

L32 ANSWER 104 OF 121 MEDLINE on STN DUPLICATE 57

1998192639. PubMed ID: 9525952. Proinflammatory cytokines regulate expression of the lymphatic endothelial mitogen vascular endothelial growth factor-C. Ristimäki A; Narko K; Enholm B; Joukov V; **Alitalo K.** (Department of Bacteriology and Immunology, the Haartman Institute, and the Department of Obstetrics and Gynecology, Haartmaninkatu 2, FIN-00290 University of Helsinki, Helsinki, Finland.) The Journal of

biological chemistry, (1998 Apr 3) Vol. 273, No. 14, pp. 8413-8. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Vascular endothelial growth factor (VEGF) is a prime regulator of normal and pathological angiogenesis. Three related endothelial cell growth factors, VEGF-B, **VEGF-C**, and VEGF-D were recently cloned. We have here studied the regulation of **VEGF-C**, a lymphatic endothelial growth factor, by angiogenic proinflammatory cytokines. Interleukin (IL)-1beta induced a concentration- and a time-dependent increase in **VEGF-C**, but not in VEGF-B, mRNA steady-state levels in human lung fibroblasts. The increase in **VEGF-C** mRNA levels was mainly due to increased transcription rather than elevated mRNA stability as detected by the nuclear run-on method and by following mRNA decay in the presence of an inhibitor of transcription, respectively. In contrast, angiopoietin-1 mRNA, encoding the ligand for the endothelial-specific Tek/Tie-2 receptor, was down-regulated by IL-1beta. Tumor necrosis factor-alpha and IL-1alpha also elevated **VEGF-C** mRNA steady-state levels, whereas the IL-1 receptor antagonist and dexamethasone inhibited the effect of IL-1beta. Experiments with cycloheximide indicated that the effect of IL-1beta was independent of protein synthesis. Hypoxia, which is an important inducer of VEGF expression, had no effect on VEGF-B or **VEGF-C** mRNA levels. IL-1beta and tumor necrosis factor-alpha also stimulated the production of **VEGF-C** protein by the fibroblasts. Cytokines and growth factors have previously been shown to down-regulate VEGF receptors in vascular endothelial cells. We found that the mRNA for the VEGF- and **VEGF-C**-binding VEGFR-2 (KDR/Flk-1) was stimulated by IL-1beta in human umbilical vein endothelial cells, whereas the mRNA levels of VEGFR-1 (Flt-1) and **VEGFR-3** (Flt-4) were not altered. Our data suggest that in addition to VEGF, **VEGF-C** may also serve as an endothelial stimulus at sites of cytokine activation. In particular, these results raise the possibility that certain proinflammatory cytokines regulate the lymphatic vessels indirectly via **VEGF-C**.

L32 ANSWER 105 OF 121 MEDLINE on STN DUPLICATE 58
1998175915. PubMed ID: 9506953. A recombinant mutant vascular endothelial growth factor-C that has lost vascular endothelial growth factor receptor-2 binding, activation, and vascular permeability activities. Joukov V; Kumar V; Sorsa T; Arighi E; Weich H; Saksela O; **Alitalo K.** (Molecular/Cancer Biology Laboratory, Haartman Institute, PL 21 Haartmaninkatu 3, University of Helsinki, 00014 Helsinki, Finland.) The Journal of biological chemistry, (1998 Mar 20) Vol. 273, No. 12, pp. 6599-602. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The vascular endothelial growth factor (VEGF) and the **VEGF-C** promote growth of blood vessels and lymphatic vessels, respectively. VEGF activates the endothelial VEGF receptors (VEGFR) 1 and 2, and **VEGF-C** activates **VEGFR-3** and VEGFR-2. Both VEGF and **VEGF-C** are also potent vascular permeability factors. Here we have analyzed the receptor binding and activating properties of several cysteine mutants of **VEGF-C** including those (Cys156 and Cys165), which in other platelet-derived growth factor/VEGF family members mediate interchain disulfide bonding. Surprisingly, we found that the recombinant mature **VEGF-C** in which Cys156 was replaced by a Ser residue is a selective agonist of **VEGFR-3**. This mutant, designated DeltaNDeltaC156S, binds and activates **VEGFR-3** but neither binds VEGFR-2 nor activates its autophosphorylation or downstream signaling to the ERK/MAPK pathway. Unlike **VEGF-C**, DeltaNDeltaC156S neither induces vascular permeability in vivo nor stimulates migration of bovine capillary endothelial cells in culture. These data point out the critical role of VEGFR-2-mediated signal

transduction for the vascular permeability activity of **VEGF-C** and strongly suggest that the redundant biological effects of VEGF and **VEGF-C** depend on binding and activation of VEGFR-2. The DeltaNDeltaC156S mutant may provide a valuable tool for the analysis of **VEGF-C** effects mediated selectively via **VEGFR-3**. The ability of DeltaNDeltaC156S to form homodimers also emphasizes differences in the structural requirements for VEGF and **VEGF-C** dimerization.

L32 ANSWER 106 OF 121 MEDLINE on STN DUPLICATE 59
1999011354. PubMed ID: 9794766. Cardiovascular failure in mouse embryos deficient in VEGF receptor-3. Dumont D J; Jussila L; Taipale J; Lymboussaki A; Mustonen T; Pajusola K; Breitman M; **Alitalo K**. (Ontario Cancer Institute and Amgen Institute, Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada M5G 2C1.) Science, (1998 Oct 30) Vol. 282, No. 5390, pp. 946-9. Journal code: 0404511. ISSN: 0036-8075. Pub. country: United States. Language: English.

AB Vascular endothelial growth factor (VEGF) is a key regulator of blood vessel development in embryos and angiogenesis in adult tissues. Unlike VEGF, the related **VEGF-C** stimulates the growth of lymphatic vessels through its specific lymphatic endothelial receptor **VEGFR-3**. Here it is shown that targeted inactivation of the gene encoding **VEGFR-3** resulted in defective blood vessel development in early mouse embryos. Vasculogenesis and angiogenesis occurred, but large vessels became abnormally organized with defective lumens, leading to fluid accumulation in the pericardial cavity and cardiovascular failure at embryonic day 9.5. Thus, **VEGFR-3** has an essential role in the development of the embryonic cardiovascular system before the emergence of the lymphatic vessels.

L32 ANSWER 107 OF 121 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
1999:6788 Document No.: PREV199900006788. Cardiovascular failure in mouse embryos deficient in VEGF receptor-3. Dumont, Daniel J.; Jussila, Lotta; Taipale, Jussi; Lymboussaki, Athina; Mustonen, Tuija; Pajusola, Katri; Breitman, Martin; **Alitalo, Kari** [Reprint author]. Sunnybrook Health Sci. Cent., Div. Cancer Biology, S Wing Res. Building, 2075 Bayview Ave., Toronto, ON M4N 3M5, Canada. Science (Washington D C), (Oct. 30, 1998) Vol. 283, No. 5390, pp. 946-949. print. CODEN: SCIEAS. ISSN: 0036-8075. Language: English.

AB Vascular endothelial growth factor (VEGF) is a key regulator of blood vessel development in embryos and angiogenesis in adult tissues. Unlike VEGF, the related **VEGF-C** stimulates the growth of lymphatic vessels through its specific lymphatic endothelial receptor **VEGFR-3**. Here it is shown that targeted inactivation of the gene encoding **VEGFR-3** resulted in defective blood vessel development in early mouse embryos. Vasculogenesis and angiogenesis occurred, but large vessels became abnormally organized with defective lumens, leading to fluid accumulation in the pericardial cavity and cardiovascular failure at embryonic day 9.5. Thus, **VEGFR-3** has an essential role in the development of the embryonic cardiovascular system before the emergence of the lymphatic vessels.

L32 ANSWER 108 OF 121 MEDLINE on STN DUPLICATE 60
1998118549. PubMed ID: 9435229. Vascular endothelial growth factor D (VEGF-D) is a ligand for the tyrosine kinases VEGF receptor 2 (Flk1) and VEGF receptor 3 (Flt4). Achen M G; Jeltsch M; Kukk E; Makinen T; Vitali A; Wilks A F; **Alitalo K**; Stacker S A. (Ludwig Institute for Cancer Research, Royal Melbourne Hospital, Victoria, Australia.. Marc.achen@ludwig.edu.au) . Proceedings of the National Academy of Sciences of the United States of America, (1998 Jan 20) Vol. 95, No. 2, pp. 548-53. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB We have identified a member of the VEGF family by computer-based homology searching and have designated it VEGF-D. VEGF-D is most closely related to **VEGF-C** by virtue of the presence of N- and C-terminal extensions that are not found in other VEGF family members. In adult human tissues, VEGF-D mRNA is most abundant in heart, lung, skeletal muscle, colon, and small intestine. Analyses of VEGF-D receptor specificity revealed that VEGF-D is a ligand for both VEGF receptors (VEGFRs) VEGFR-2 (Flk1) and **VEGFR-3** (Flt4) and can activate these receptors. However, VEGF-D does not bind to VEGFR-1. Expression of a truncated derivative of VEGF-D demonstrated that the receptor-binding capacities reside in the portion of the molecule that is most closely related in primary structure to other VEGF family members and that corresponds to the mature form of **VEGF-C**. In addition, VEGF-D is a mitogen for endothelial cells. The structural and functional similarities between VEGF-D and **VEGF-C** define a subfamily of the VEGFs.

L32 ANSWER 109 OF 121 MEDLINE on STN DUPLICATE 61
 1999023338. PubMed ID: 9808152. Vascular endothelial growth factor (**VEGF**)-C synergizes with basic fibroblast growth factor and VEGF in the induction of angiogenesis in vitro and alters endothelial cell extracellular proteolytic activity. Pepper M S; Mandriota S J; Jeltsch M; Kumar V; **Alitalo K.** (Department of Morphology, University Medical Center, Geneva, Switzerland.. michael.pepper@medecine.unige.ch) . Journal of cellular physiology, (1998 Dec) Vol. 177, No. 3, pp. 439-52. Journal code: 0050222. ISSN: 0021-9541. Pub. country: United States. Language: English.

AB Vascular endothelial growth factor-C (**VEGF-C**) is a recently characterized member of the VEGF family of angiogenic polypeptides. We demonstrate here that **VEGF-C** is angiogenic in vitro when added to bovine aortic or lymphatic endothelial (BAE and BLE) cells but has little or no effect on bovine microvascular endothelial (BME) cells. As reported previously for VEGF, **VEGF-C** and basic fibroblast growth factor (bFGF) induced a synergistic in vitro angiogenic response in all three cell lines. Unexpectedly, VEGF and **VEGF-C** also synergized in the in vitro angiogenic response when assessed on BAE cells. Characterization of VEGF receptor (VEGFR) expression revealed that BME, BAE, and BLE cell lines express VEGFR-1 and -2, whereas of the three cell lines assessed, only BAE cells express **VEGFR-3**. We also demonstrate that **VEGF-C** increases plasminogen activator (PA) activity in the three bovine endothelial cell lines and that this is accompanied by a concomitant increase in PA inhibitor-1. Addition of alpha2-antiplasmin to BME cells co-treated with bFGF and **VEGF-C** partially inhibited collagen gel invasion. These results demonstrate, first, that by acting in concert with bFGF or VEGF, **VEGF-C** has a potent synergistic effect on the induction of angiogenesis in vitro and, second, that like VEGF and bFGF, **VEGF-C** is capable of altering endothelial cell extracellular proteolytic activity. These observations also highlight the notion of context, i.e., that the activity of an angiogenesis-regulating cytokine depends on the presence and concentration of other cytokines in the pericellular environment of the responding endothelial cell.

L32 ANSWER 110 OF 121 MEDLINE on STN DUPLICATE 62
 1998372531. PubMed ID: 9708800. Expression of the vascular endothelial growth factor C receptor **VEGFR-3** in lymphatic endothelium of the skin and in vascular tumors. Lymboussaki A; Partanen T A; Olofsson B; Thomas-Crusells J; Fletcher C D; de Waal R M; Kaipainen A; **Alitalo K.** (Department of Pathology, Haartman Institute, University of Helsinki, Finland.) The American journal of pathology, (1998 Aug) Vol. 153, No. 2, pp. 395-403. Journal code: 0370502. ISSN: 0002-9440. Pub. country: United States. Language: English.

AB It is difficult to identify lymph vessels in tissue sections by histochemical staining, and thus a specific marker for lymphatic endothelial cells would be more practical in histopathological diagnostics. Here we have applied a specific antigenic marker for lymphatic endothelial cells in the human skin, the vascular endothelial growth factor receptor-3 (**VEGFR-3**), and show that it identifies a distinct vessel population both in fetal and adult skin, which has properties of lymphatic vessels. The expression of **VEGFR-3** was studied in normal human skin by in situ hybridization, iodinated ligand binding, and immunohistochemistry. A subset of developing vessels expressed the **VEGFR-3** mRNA in fetal skin as shown by in situ hybridization and radioiodinated vascular endothelial growth factor (**VEGF**)-C bound selectively to a subset of vessels in adult skin that had morphological characteristics of lymphatic vessels. Monoclonal antibodies against the extracellular domain of **VEGFR-3** stained specifically endothelial cells of dermal lymph vessels, in contrast to PAL-E antibodies, which stained only blood vessel endothelia. In addition, staining for **VEGFR-3** was strongly positive in the endothelium of cutaneous lymphangiomatosis, but staining of endothelial cells in cutaneous hemangiomas was weaker. These results establish the utility of anti-**VEGFR-3** antibodies in the identification of lymphovascular channels in the skin and in the differential diagnosis of skin lesions involving lymphatic or blood vascular endothelium.

L32 ANSWER 111 OF 121 MEDLINE on STN DUPLICATE 63
2004098748. PubMed ID: 14987553. Vascular endothelial growth factor-C: a growth factor for lymphatic and blood vascular endothelial cells. Enholm B; Jussila L; Karkkainen M; **Alitalo K.** (Molecular/Cancer Biology Laboratory, Haartman Institute, University of Helsinki, Finland.) Trends in cardiovascular medicine, (1998 Oct) Vol. 8, No. 7, pp. 292-7. Journal code: 9108337. ISSN: 1050-1738. Pub. country: United States. Language: English.

AB The endothelial cells lining all vessels of the circulatory system have been recognized as key players in a variety of physiological and pathological settings. They act as regulators of vascular tone via the inducible nitric oxide system and in angiogenesis, the formation of blood vessels de novo. Aberrant regulation of endothelial cells contributes to tumor formation, atherosclerosis, and diseases such as psoriasis and rheumatoid arthritis. Among the most recently discovered growth factors for endothelial cells are newly isolated members of the platelet-derived growth factor/vascular endothelial growth factor (VEGF) family, VEGF-B, **VEGF-C**, and VEGF-D. **VEGF-C** is the ligand for the receptor tyrosine kinase **VEGFR-3** (also known as Flt4), which is expressed predominantly in lymphatic endothelium of adult tissues, but a proteolytically processed form of **VEGF-C** can also activate VEGFR-2 of blood vessels. The lymphatic vessels have been known since the 17th century, but their specific roles in health and disease are still poorly understood. With the discovery of **VEGF-C** and its cognate receptor **VEGFR-3**, the regulation and functions of this important component of the circulatory system can be investigated.

L32 ANSWER 112 OF 121 MEDLINE on STN DUPLICATE 64
97377029. PubMed ID: 9233800. Proteolytic processing regulates receptor specificity and activity of **VEGF-C**. Joukov V; Sorsa T; Kumar V; Jeltsch M; Claesson-Welsh L; Cao Y; Saksela O; Kalkkinen N; **Alitalo K.** (Molecular/Cancer Biology Laboratory, University of Helsinki, Finland.) The EMBO journal, (1997 Jul 1) Vol. 16, No. 13, pp. 3898-911. Journal code: 8208664. ISSN: 0261-4189. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The recently identified vascular endothelial growth factor C (**VEGF**

-C) belongs to the platelet-derived growth factor (PDGF)/VEGF family of growth factors and is a ligand for the endothelial-specific receptor tyrosine kinases **VEGFR-3** and **VEGFR-2**. The VEGF homology domain spans only about one-third of the cysteine-rich **VEGF-C** precursor. Here we have analysed the role of post-translational processing in **VEGF-C** secretion and function, as well as the structure of the mature **VEGF-C**. The stepwise proteolytic processing of **VEGF-C** generated several **VEGF-C** forms with increased activity towards **VEGFR-3**, but only the fully processed **VEGF-C** could activate **VEGFR-2**. Recombinant 'mature' **VEGF-C** made in yeast bound **VEGFR-3** ($K[D] = 135 \text{ pM}$) and **VEGFR-2** ($K[D] = 410 \text{ pM}$) and activated these receptors. Like VEGF, mature **VEGF-C** increased vascular permeability, as well as the migration and proliferation of endothelial cells. Unlike other members of the PDGF/VEGF family, mature **VEGF-C** formed mostly non-covalent homodimers. These data implicate proteolytic processing as a regulator of **VEGF-C** activity, and reveal novel structure-function relationships in the PDGF/VEGF family.

- L32 ANSWER 113 OF 121 MEDLINE on STN DUPLICATE 65
 97306401. PubMed ID: 9162011. Hyperplasia of lymphatic vessels in **VEGF-C** transgenic mice. Jeltsch M; Kaipainen A; Joukov V; Meng X; Lakso M; Rauvala H; Swartz M; Fukumura D; Jain R K; **Alitalo K**. (Molecular/Cancer Biology Laboratory, Haartman Institute, University of Helsinki, Finland.) Science, (1997 May 30) Vol. 276, No. 5317, pp. 1423-5. Journal code: 0404511. ISSN: 0036-8075. Pub. country: United States. Language: English.
- AB No growth factors specific for the lymphatic vascular system have yet been described. Vascular endothelial growth factor (VEGF) regulates vascular permeability and angiogenesis, but does not promote lymphangiogenesis. Overexpression of **VEGF-C**, a ligand of the VEGF receptors **VEGFR-3** and **VEGFR-2**, in the skin of transgenic mice resulted in lymphatic, but not vascular, endothelial proliferation and vessel enlargement. Thus, **VEGF-C** induces selective hyperplasia of the lymphatic vasculature, which is involved in the draining of interstitial fluid and in immune function, inflammation, and tumor metastasis. **VEGF-C** may play a role in disorders involving the lymphatic system and may be of potential use in therapeutic lymphangiogenesis.
- L32 ANSWER 114 OF 121 MEDLINE on STN DUPLICATE 66
 97392771. PubMed ID: 9245515. VEGF and **VEGF-C**: specific induction of angiogenesis and lymphangiogenesis in the differentiated avian chorioallantoic membrane. Oh S J; Jeltsch M M; Birkenhager R; McCarthy J E; Weich H A; Christ B; **Alitalo K**; Wilting J. (Anatomisches Institut II, Albert-Ludwigs-Universitat Freiburg, Albertstrasse 17, Freiburg, D-79104, Germany.) Developmental biology, (1997 Aug 1) Vol. 188, No. 1, pp. 96-109. Journal code: 0372762. ISSN: 0012-1606. Pub. country: United States. Language: English.
- AB The lymphangiogenic potency of endothelial growth factors has not been studied to date. This is partially due to the lack of in vivo lymphangiogenesis assays. We have studied the lymphatics of differentiated avian chorioallantoic membrane (CAM) using microinjection of Mercox resin, semi- and ultrathin sectioning, immunohistochemical detection of fibronectin and alpha-smooth muscle actin, and in situ hybridization with **VEGFR-2** and **VEGFR-3** probes. CAM is drained by lymphatic vessels which are arranged in a regular pattern. Arterioles and arteries are accompanied by a pair of interconnected lymphatics and form a plexus around bigger arteries. Veins are also associated with lymphatics, particularly larger veins, which are surrounded by a lymphatic plexus. The lymphatics are characterized by an

extremely thin endothelial lining, pores, and the absence of a basal lamina. Patches of the extracellular matrix can be stained with an antibody against fibronectin. Lymphatic endothelial cells of differentiated CAM show ultrastructural features of this cell type. CAM lymphatics do not possess mediae. In contrast, the lymphatic trunks of the umbilical stalk are invested by a single but discontinuous layer of smooth muscle cells. CAM lymphatics express VEGFR-2 and **VEGFR-3**. Both the regular pattern and the typical structure of these lymphatics suggest that CAM is a suitable site to study the in vivo effects of potential lymphangiogenic factors. We have studied the effects of VEGF homo- and heterodimers, VEGF/PlGF heterodimers, and PlGF and **VEGF-C** homodimers on Day 13 CAM. All the growth factors containing at least one VEGF chain are angiogenic but do not induce lymphangiogenesis. PlGF-1 and PlGF-2 are neither angiogenic nor lymphangiogenic. **VEGF-C** is the first lymphangiogenic factor and seems to be highly chemoattractive for lymphatic endothelial cells. It induces proliferation of lymphatic endothelial cells and development of new lymphatic sinuses which are directed immediately beneath the chorionic epithelium. Our studies show that VEGF and **VEGF-C** are specific angiogenic and lymphangiogenic growth factors, respectively.

L32 ANSWER 115 OF 121 MEDLINE on STN DUPLICATE 67
 97164697. PubMed ID: 9012504. **VEGF-C** receptor binding and pattern of expression with **VEGFR-3** suggests a role in lymphatic vascular development. Kukk E; Lymboussaki A; Taira S; Kaipainen A; Jeltsch M; Joukov V; **Alitalo K.** (Molecular/Cancer Biology Laboratory, Haartman Institute, University of Helsinki, Finland.) Development (Cambridge, England), (1996 Dec) Vol. 122, No. 12, pp. 3829-37. Journal code: 8701744. ISSN: 0950-1991. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The vascular endothelial growth factor family has recently been expanded by the isolation of two new VEGF-related factors, VEGF-B and **VEGF-C**. The physiological functions of these factors are largely unknown. Here we report the cloning and characterization of mouse **VEGF-C**, which is produced as a disulfide-linked dimer of 415 amino acid residue polypeptides, sharing an 85% identity with the human **VEGF-C** amino acid sequence. The recombinant mouse **VEGF-C** protein was secreted from transfected cells as **VEGFR-3** (Flt4) binding polypeptides of 30-32x10(3) Mr and 22-23x10(3) Mr which preferentially stimulated the autophosphorylation of **VEGFR-3** in comparison with VEGFR-2 (KDR). In situ hybridization, mouse **VEGF-C** mRNA expression was detected in mesenchymal cells of postimplantation mouse embryos, particularly in the regions where the lymphatic vessels undergo sprouting from embryonic veins, such as the perimetanephric, axillary and jugular regions. In addition, the developing mesenterium, which is rich in lymphatic vessels, showed strong **VEGF-C** expression. **VEGF-C** was also highly expressed in adult mouse lung, heart and kidney, where **VEGFR-3** was also prominent. The pattern of expression of **VEGF-C** in relation to its major receptor **VEGFR-3** during the sprouting of the lymphatic endothelium in embryos suggests a paracrine mode of action and that one of the functions of **VEGF-C** may be in the regulation of angiogenesis of the lymphatic vasculature.

L32 ANSWER 116 OF 121 MEDLINE on STN
 96203094. PubMed ID: 8612600. A novel vascular endothelial growth factor, **VEGF-C**, is a ligand for the Flt4 (**VEGFR-3**) and KDR (**VEGFR-2**) receptor tyrosine kinases. Joukov V; Pajusola K; Kaipainen A; Chilov D; Lahtinen I; Kukk E; Saksela O; Kalkkinen N; **Alitalo K.** The EMBO journal, (1996 Apr 1) Vol. 15, No. 7, pp. 1751. Journal code: 8208664. ISSN: 0261-4189. Pub. country: ENGLAND:

United Kingdom. Language: English.

L32 ANSWER 117 OF 121 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN

96118150 EMBASE Document No.: 1996118150. Erratum: A novel vascular endothelial growth factor, **VEGF-C**, is a ligand for the Flt4 (**VEGFR-3**) and KDR (VEGFR-2) receptor tyrosine kinases (The EMBO Journal (1996) 15 (290-298)). Jsukov V.; Pajusola K.; Kaipainen A.; Chilov D.; Lahtinen I.; Kukkk E.; Saksela O.; Kalkkinen N.; **Alitalo K.** EMBO Journal Vol. 15, No. 7, pp. 1751 1996. ISSN: 0261-4189. CODEN: EMJODG
Pub. Country: United Kingdom. Language: English.
Entered STN: 960507. Last Updated on STN: 960507
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L32 ANSWER 118 OF 121 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

1996:293613 The Genuine Article (R) Number: UF658. A novel vascular endothelial growth factor, **VEGF-C**, is a ligand for the Flt4 (**VEGFR-3**) and KDR (VEGFR-2) receptor tyrosine kinases (vol 15, pg 290, 1996). Joukov V (Reprint); Pajusola K; Kaipainen A; Chilov D; Lahtinen I; Kukkk E; Saksela O; Kalkkinen N; **Alitalo K** . EMBO JOURNAL (1 APR 1996) Vol. 15, No. 7, pp. 1751-1751. ISSN: 0261-4189 . Publisher: OXFORD UNIV PRESS UNITED KINGDOM, WALTON ST JOURNALS DEPT, OXFORD, ENGLAND OX2 6DP. Language: English.

L32 ANSWER 119 OF 121 CAPLUS COPYRIGHT 2006 ACS on STN

1996:247586 Document No. 125:2261 A novel vascular endothelial growth factor, **VEGF-C**, is a ligand for the Flt4 (**VEGFR-3**) and KDR (VEGFR-2) receptor tyrosine kinases. [Erratum to document cited in CA124:136478]. Joukov, Vladimir; Pajusola, Katri; Kaipainen, Arja; Chilov, Dmitri; Lahtinen, Isto; Kukkk, Eola; Saksela, Olli; Kalkkinen, Nisse; **Alitalo, Kari** (Dep. Virology, Univ. Helsinki, Helsinki, 0014, Finland). EMBO Journal, 15(7), 1751 (English) 1996. CODEN: EMJODG. ISSN: 0261-4189. Publisher: Oxford University Press.

AB The errors were not reflected in the abstract The sequence of the reported vascular endothelial growth factor **VEGF-C** was corrected to show an addnl. 69 amino acids at the N-terminus of the protein. **VEGF-C** thus exists as a preproprotein rather than a simple precursor.

L32 ANSWER 120 OF 121 MEDLINE on STN

DUPLICATE 68

96178224. PubMed ID: 8617204. A novel vascular endothelial growth factor, **VEGF-C**, is a ligand for the Flt4 (**VEGFR-3**) and KDR (VEGFR-2) receptor tyrosine kinases. Joukov V; Pajusola K; Kaipainen A; Chilov D; Lahtinen I; Kukkk E; Saksela O; Kalkkinen N; **Alitalo K.** (Department of Virology, Haartman Institute, University of Helsinki, Finland.) The EMBO journal, (1996 Jan 15) Vol. 15, No. 2, pp. 290-98. Journal code: 8208664. ISSN: 0261-4189. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Angiogenesis, the sprouting of new blood vessels from pre-existing ones, and the permeability of blood vessels are regulated by vascular endothelial growth factor (VEGF) via its two known receptors Flt1 (VEGFR-1) and KDR/Flk-1 (VEGFR-2). The Flt4 receptor tyrosine kinase is related to the VEGF receptors, but does not bind VEGF and its expression becomes restricted mainly to lymphatic endothelia during development. In this study, we have purified the Flt4 ligand, **VEGF-C**, and cloned its cDNA from human prostatic carcinoma cells. While **VEGF-C** is homologous to other members of the VEGF/platelet derived growth factor (PDGF) family, its C-terminal half contains extra cysteine-rich motifs characteristic of a protein component of silk produced by the larval salivary glands of the midge, Chironomus

tentans. **VEGF-C** is proteolytically processed, binds Flt4, which we rename as **VEGFR-3** and induces tyrosine autophosphorylation of **VEGFR-3** and VEGFR-2. In addition, **VEGF-C** stimulated the migration of bovine capillary endothelial cells in collagen gel. **VEGF-C** is thus a novel regulator of endothelia, and its effects may extend beyond the lymphatic system, where Flt4 is expressed.

L32 ANSWER 121 OF 121 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

2000:502196 Document No.: PREV200000487774. **VEGF-C**, VEGF-D and **VEGFR-3** in tumor angiogenesis, lymphangiogenesis and metastasis. **Alitalo, K.** [Reprint author]. Molecular/Cancer Biology Laboratory, Haartman Institute, University of Helsinki, Helsinki, Finland. Clinical and Experimental Metastasis, (1999 (2000)) Vol. 17, No. 9, pp. 740. print.

Meeting Info.: VIII International Congress of the Metastasis Research Society. London, UK. September 24-27, 2000.

CODEN: CEXMD2. ISSN: 0262-0898. Language: English.

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